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CYTOGENETIC EVOLUTIONARY PROCESSES AND THEIR BEARING ON EVOLUTIONARY THEORY

CYTOGENETIC EVOLUTIONARY PROCESSES IN PLANTS[1, 2]

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THE relationship between cytology and genetics with reference to certain problems of heredity and evolution has become increasingly intimate in recent years. The two disciplines, indeed, have come to occupy in part a common ground, which is appropriately termed cytogenetics. It is my present purpose to consider some of the findings in the botanical portion of this field in their bearing on evolution.

By definition the discussion is limited to those processes of which some direct evidence may be obtained with the microscope. This excludes what appears to be the primary factor in evolution, namely, gene mutation. In spite of the extremely unfavorable nature of the material which the process ordinarily displays, the conclusion seems inescapable that the basic element in evolution is gene change. We are impelled to this view, however, not by any direct evidence of contemporary progressive evolution by this means, but rather by the facts of

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genetics. If the gene has proved to be the ultimate unit of heredity, by the same token, it is very probably the ultimate unit of evolution. I do not think that the logic of this inference can be refuted. The deductive origin of the conclusion implies, however, that our knowledge of the gene as a factor in evolution is restricted in large part to what we know of it as a hereditary unit. The limitation is an important one. Genetics treats of the gene essentially as a stable thing; evolution demands, in the first instance, a knowledge of its mutability. While important advances in the field have been made in recent years, gene mutation is still a very obscure process. Attempts to connect the scattered evidence regarding the rôle of gene mutation in evolution are being undertaken profitably at the present time, but it must be frankly admitted that in coordinating the facts numerous wide gaps of ignorance are encountered which are only to be bridged with the tenuous thread of speculation.

When we turn to a consideration of certain evolutionary processes of higher order the case, I think, is somewhat better. Investigations of the last decade or so have revealed some grosser types of changes in the germplasm of indubitable importance for evolution. It may even be claimed that the proof of evolutionary significance of certain of them is complete if the evidence required is the production of forms which may be ranked as new species.

It is proposed to discuss three general classes of cytogenetic processes which have been the objects of considerable study and which appear to be of significance in evolution: (a) amphidiploidy, (b) changes in chromosome number not involving the whole genom and (c) structural changes in the chromosomes. The general plan of presentation will be first, to point out briefly the nature of the evidence indicating that the process in question actually has played a rôle in the differentiation of existing species. Secondly, some of the results which detailed experimental analysis of the phenomenon has afforded

will be reviewed. Where the synthesis under controlled conditions of types which, in certain respects at least, meet the requirements of new species has been accomplished, the fact will be mentioned.

New Chromosome Combinations

A cytogenetic phenomenon which is of evolutionary importance but which is not covered by the above classification is the formation of new chromosome combinations following interspecific hybridization. J. Clausen's (1931) extensive work on the Melanium violets affords some instructive examples. The phylogeny of this group is very complex and, according to Clausen, is to be represented as an intricate network rather than as a branching tree. In other words, through an interlacing of the ancestral lines the species have derived the components of their genoms from diverse sources. Pedigree culture studies of controlled species crosses bear out this hypothesis. For example, from a hybrid between *Viola tricolor* ($n=13$) and *V. orphanidis* ($n=11$) there was derived a new, constant and vigorous form with ($n=13$) chromosomes which Clausen terms *V. crassicaulis*. It has the chromosome number of its *tricolor* parent, but is morphologically distinct from it. *V. crassicaulis* is sufficiently vigorous and fertile to be conceded a change in nature, and if found there would doubtless be accorded specific rank. There is good reason for thinking that many of the species of *Viola* have arisen in this way.

Heribert Nilsson's (1930) studies point to a similar method of origin of certain *Salix* species. In this genus, which is also taxonomically very difficult, interspecific hybridization appears to be common in nature. With the direct evidence now available there can be little doubt of the importance of species crossing as a means by which new forms of *Salix* have originated. It is to be recognized, of course, that following species hybridization the way is opened to various phenomena of evolutionary significance. In the present connection it is in-

tended to call attention particularly to the possibilities presented merely for segregation and the formation of new combinations.

AMPHIDIPLOIDY

The simple numerical relationships between the chromosome numbers in the species of certain genera, particularly in the Angiosperms, early attracted the attention of cytologists. Winge (1917) pointed out that among the gametic numbers there was a relatively high frequency of multiples. A considerably larger body of data has since become available for examination. Fernandes (1931) drew up a frequency table showing the distribution of 2,413 species with reference to chromosome number between the limits 3 and 100. The striking fact emerged that there is not a single mode at a prime number, *viz.*, 7, 11, 13, 17, 19, etc. Furthermore, the most frequent classes are those whose values have the lowest factors, *viz.*, 8, 12, 16, etc. These crude statistical data alone create a strong presumption in favor of the view that, in the evolution of the Angiosperms, species with chromosome numbers in multiple series are favored, either with respect to origin or persistence. When the further fact is considered that there are numerous genera such as *Rosa* and *Chrysanthemum,* in which the chromosome number varies only in terms of some basic value, it becomes highly probable *a priori* that in the flowering plants this phenomenon, which is termed polyploidy, has been of far-reaching evolutionary significance. Since other studies indicated no grounds for thinking that particular chromosome numbers, *per se,* bear any relation to survival value attention was directed to polyploidy as a means by which new species originate.

An hypothesis to account for the derivation of species possessing chromosome numbers in polyploid series was brought forward by Winge in 1917. It has since received striking confirmation in the work of several investigators.

One might venture the remark in passing that the discovery of amphidiploidy and the clear-cut demonstration under controlled conditions of the origin by this means of forms meeting the requirements of new species marked a turning point in the relation between genetics and evolutionary theory. Those in the first decades of the present century who maintained high hopes that the ingenious new science of heredity was to provide the keys to the mechanism of evolution, only to experience a growing doubt as the years passed by without a lock being forced, were at last vindicated. Here, indeed, genetics with the indispensable aid of its sister discipline, cytology, had gained admittance to one of the tool chests of evolution. If the contents of the others still seemed rather mysterious, no matter; there was definite evidence that we were on the right track.

The essential feature of Winge's hypothesis on the origin of species with chromosome numbers in polyploid series is familiar. Suppose two species, *A* and *B*, in a particular genus each possess a given gametic number of chromosomes, say 7. Each chromosome of the one set is assumed to be so differentiated in content of genes from the corresponding chromosome of the other set that conjugation in a hybrid would not occur. If species *A* and *B* now cross, the resulting zygote will be sterile on account of the incompatibility of the two genoms. Should this chromosome complement become doubled, however, each chromosome would have a mate, regular pairing would be possible, and fertility would ensue. The new type would contain the genoms of both the parent forms, hence the term amphidiploid, and would itself rank as a new species.

Confirmation of Winge's hypothesis was first afforded by the experimental production by Clausen and Goodspeed (1925) of the amphidiploid, *Nicotiana digluta.* The one parent, *N. glutinosa,* has 12 pairs of chromosomes and the other, *N. tabacum,* which is very distinct, has 24. The two species when crossed give a weak

hybrid which is only slightly fertile. Clausen and Goodspeed raised an F_2 family of 65 individuals from the seed in a single capsule of such a partially fertile *N. glutinosa* × *N. tabacum* hybrid, and found the plants remarkably uniform and closely resembling the F_1, except that they were much more fertile. Cytological examinations showed that they possessed 36 pairs of chromosomes, the sum of the numbers of the two parental species. *N. digluta* is regular in its meiotic behavior and breeds true.

Abundant further evidence of the origin of new forms by amphidiploidy has since been secured. The intergeneric hybrid between the radish and the cabbage has yielded the true-breeding *Raphano-Brassica* (Karpechenko, 1927), and that between *Triticum durum* and *Aegilops ovata* has given *Aegilotricum* (Tschermak and Bleier, 1926). Other amphidiploid species which have originated under controlled conditions are *Digitalis Mertonensis* (Buxton and Newton, 1928) from *D. purpurea* and *D. ambigua,* a Nicotiana with 36 pairs of chromosomes from *N. tabacum* × *N. sylvestris* (Rybin, 1929) and *Primula Kewensis* from *P. floribunda* and *P. verticillata* (Newton and Pellew, 1929). The list might be further extended.

It has been shown that there are two principal ways in which chromosome doubling takes place spontaneously in the formation of amphidiploids, *viz.,* in somatic tissue, and through the formation of restitution nuclei (Rosenberg, 1917) at meiosis, leading to the development of unreduced gametes. The latter mode of origin appears to be the more common.

A notable feature of several experimentally produced amphidiploid hybrids is their comparatively high fertility and constancy. The two, of course, are closely associated and are determined to a large extent by the course of the meiotic divisions. There is a negative correlation, although not an absolute one, between the degree of chromosome pairing in the original species

hybrid and the regularity of meiotic behavior in the derived amphidiploid. If the chromosomes of the two respective genoms show little tendency to conjugate in the initial hybrid it is likely that the meiotic divisions in the amphidiploid will be undisturbed by the formation of multivalents. This is the case in *Raphano-Brassica*, *Nicotiana digluta* and *Aegilotrichum*. In *Crepis rubra* × *C. foetida* (Poole, 1931), on the other hand, pairing is fairly regular in the F_1 primary hybrid; and quadrivalents are frequently formed in the amphidiploid. Considerable sterility results. In *Solanum nigrum* × *S. luteum* and in *Primula Kewensis*, however, the primary hybrids show more or less regular pairing, yet in spite of this the amphidiploid forms show relatively few quadrivalents. There appears to be no satisfactory explanation for the exceptional cases. As would be expected, the degree of constancy of the artificially produced amphidiploids is parallel to the regularity of their meiotic behavior.

The amphidiploids discussed thus far have all arisen under experimental conditions, and are not known to occur in the wild. Proof of the significance for evolution of species hybridization followed by chromosome doubling would be complete if it could be shown that certain established species must have originated in this way. Some very cogent evidence on this point is at hand, a part of which is the result of a method of approach to the problem which has been termed genom analysis. The procedure is applicable in cases where the genom of the amphidiploid species under investigation can be brought into combination in a hybrid with the chromosome complements of each of the assumed parents. The criterion which is then applied is the capacity of the apposed chromosomes to conjugate in meiosis. The validity of this test has been questioned by Darlington (1932, p. 459) on the grounds that differences in the chromosomes which would be expected to reduce pairing are not necessarily directly related to

the differentiation of the genoms in genetic properties. There is reason for thinking that not infrequently in species hybrids such a situation may obtain, so that the criterion is not to be considered infallible. Nevertheless, in many cases, pairing doubtless serves as a rather accurate index of homology.

Using this criterion of homology, Clausen (1928, 1932) and Goodspeed and Clausen (1928) have been able to show that *Nicotiana tabacum* ($n=24$) probably originated by chromosome doubling in a hybrid between *N. sylvestris* ($n=12$) and *N. tomentosa* ($n=12$) (or *N. tomentosiformis* = *N. Rusbyi*), or close allies of these species. The haploid *tabacum* displays no conjugation of the chromosomes in meiosis, nor does the hybrid, *sylvestris* × *tomentosa*. The F_1 hybrids, *sylvestris* × *tabacum*, and *tomentosa* × *tabacum* on the other hand, exhibit 12 bivalent chromosomes and 12 univalent chromosomes in the first meiotic metaphase. Presumably the 12 chromosomes of *tabacum* with which the *sylvestris* set pairs are different from those with which the *tomentosa* genom conjugates. Some direct evidence that this is actually the case has been obtained. Clausen (1932) has shown, for example, that the *sylvestris* subgenom of *tabacum* consists mainly of large chromosomes, while the *tomentosa* group is made up mainly of smaller ones. By the ingenious use in hybrids with *sylvestris* and with *tomentosa* of certain monosomic ($2n-1$) testers isolated from *tabacum*, Clausen, likewise, has been able to assign three specific *tabacum* chromosomes to their respective subgenoms. Further evidence that *tabacum* is derived from a hybrid between *sylvestris* and *tomentosa*, or their close relatives, is afforded by the independent investigations of Brieger (1930) and Kostoff (1931). Each of these workers obtained in a different way trigenomatic plants carrying the genoms of *sylvestris*, *tomentosiformis* and *tabacum*, and found that they formed 24 bivalent chromosomes in meiosis as required by hypothesis.

Galeopsis Tetrahit

Evidence of another sort that certain existing species have arisen in nature by hybridization followed by chromosome doubling is provided by Müntzing's (1932) artificial synthesis of *Galeopsis Tetrahit,* the hemp nettle. Crosses between *G. pubescens* and *G. speciosa,* both of which have 8 as the haploid chromosome number, are highly sterile in F_1. Among about 200 F_2 individuals which Müntzing reared one plant proved to be triploid and almost sterile. A single seed was obtained on pollinating it with *G. pubescens.* This seed gave a fertile tetraploid plant which became the progenitor of the line which is termed artificial *G. Tetrahit.* Morphologically, cytologically and in its genetic behavior, artificial Tetrahit is indistinguishable from the species *G. Tetrahit.* Müntzing considers the fact established that the wild form represents a synthesis of the *pubescens* and *speciosa* genoms, and presents the case as the first example of an established species which has been produced synthetically from two other species.

Spartina Townsendii

If evidence is demanded as to the capability of amphidiploid species superseding their ancestral forms the interesting case of *Spartina,* Cord or Rice Grass, which Huskins (1931) has recently reported, may be cited. It was shown that *Spartina Townsendii* has $2n = 126$ chromosomes, the sum of the numbers occurring in *S. stricta* ($2n = 56$) and *S. alterniflora* ($2n = 70$). This fact, its fertility and the circumstances of its distribution are plausible reasons for thinking that *S. Townsendii* is an amphidiploid derivate of the other two species. *S. stricta* has long been known as occurring on certain parts of the Atlantic coast of Europe. *S. alterniflora,* on the other hand, is believed to be of American origin, and to have been carried to Europe with shipping. According to Huskins, it was first recorded in Europe at Bayonne in 1803 and at Southampton in 1829. Fifty years ago

Townsend observed that *stricta* was rather common along the Hampshire coast in England, *alterniflora* was rather rare and *Townsendii* was rare, except in one locality. To-day, however, *Townsendii,* which is a vigorous and aggressive species, has spread along the coast of England and has crossed the channel. Where the two come in contact, *Townsendii* is eliminating its one parent, *stricta,* while *alterniflora* remains definitely restricted in its distribution.

Penstemon neotericus ($n = 32$), which J. Clausen (1933) has shown to have arisen, probably by amphidiploidy from *P. laetus* ($n = 8$) and *P. azureus* ($n = 24$), is another case in point. The two latter species overlap in their distribution in the Sierra Nevada, and *neotericus,* which represents a combination of characters found in these forms, occupies a vacant area adjacent to them. In this example, as in that of *Spartina,* the amphidiploid has not been produced experimentally. The morphological and cytological findings, pointing to an amphidiploid origin, however, are highly suggestive, even if not conclusive. The evidence from field studies as to the success of the species in nature is incontestable.

An obscure point in connection with the origin of amphidiploid species is the nature of the differentiation between the chromosomes in the two parent genoms which leads to failure of pairing in the primary hybrid. Is this differentiation structural? In other words, does it rest upon variation in position of chromosome segments which in their total gene content are not greatly unlike; or is it due to allelomorphic differences at many loci whose respective positions in the complement have not been altered? This question, which is merely a phase of the broader one regarding the causes of the pairing of synaptic mates, must go unanswered.

Amphidiploidy involves the production of species with numbers of chromosomes in higher multiples than those of the parent forms. This has been thought of as essentially an irreversible process. Doubtless the change is

usually to a greater chromosome number. As Dusseau (1932) and Nishiyama (1933) have recently shown, however, the process may go in the opposite direction on occasion, *i.e.,* the number of chromosome sets in the derived form may be less than that in a parent type. Dusseau (1932) reports the occurrence of a new fertile diploid wheat with ($2n = 14$) chromosomes among the offspring of an intervarietal hybrid in the hexaploid species, *Triticum vulgare* ($2n = 42$). Nishiyama crossed the hexaploid, *Avena fatua* ($2n = 42$), with the tetraploid, *A. barbata* ($2n = 28$), and in the progeny of one F_2 derivative with 42 chromosomes a ($2n = 14$) diploid was found. The plant regularly formed seven bivalents and was fertile. It was distinctly different from the mother plant and from the parental species and bred true for chromosome number, although it segregated for some morphological characters. It also differed more or less from the known diploid species of Avena, with which it could be readily hybridized.

Müntzing (1934) suggests that in species hybrids chromosomes which are structurally differentiated, but which contain homologous segments, may cross over and give rise to new chromosome types of possible evolutionary value. He finds support for this conception in the results of a study of a hybrid between *Crepis divaricata* and *C. dioscorides,* two species belonging to different subdivisions of the genus. While the number of chromosomes in both is the same, $n = 4$, the chromosomes differ in their morphology. At metaphase I in the hybrid 0–4 bivalents occur, some of which at least are probably held together by interstitial chiasmata. It is assumed that this association is made possible by partial homology of the chromosomes, *i.e.,* some segments of the chromosomes are homologous, while others are not. Fragments are frequently observed in metaphase and anaphase and also chromosomes with two spindle fiber attachments. Crossing over would be expected to yield products of these kinds. Presumably chromosomes also

arise with only a single spindle fiber attachment but differing structurally from those in the parental species. The possibility is thus presented for the formation of new genoms, some of which might be expected to have evolutionary value.

CHANGES IN CHROMOSOME NUMBER NOT INVOLVING THE WHOLE GENOM

Of more fundamental evolutionary significance than amphidiploidy, probably, is the process or processes by which changes in chromosome number occur which do not involve the whole genom. Numerous examples might be cited of genera in which the chromosome numbers tend to run not in multiples, but in longer or shorter arithmetical series. Heilborn's (1932) work on the sedge, *Carex,* furnishes a familiar example of extreme variation of this kind. Species are known in *Carex* with the following chromosome numbers: $n = 9$, 15, 16, 18, 19, every number from 23 to 43, and 56. The problem which is presented here has been attacked along broad lines in recent years, and while the results achieved are much less definite in character than in the case of amphidiploid species, significant progress has been made. Our present knowledge regarding the mode of origin of species with aneuploid chromosome numbers is based in part on inferences drawn from studies in comparative karyology. In passing, it is worth while to emphasize the difficulties confronting the investigator in this field and the limitations of the method.

The principal criterion of the taxonomist in constructing his schemes of phylogenetic relationship is morphological differentiation. Genetics has served to show that these morphological characteristics have their basis in the genes and that the latter are organized into linkage groups corresponding to the haploid number of chromosomes characteristic of the species. The point of departure in studies on comparative karyology is the significant fact that when the genoms of a group of related

organisms are compared similarities of organization are not infrequently evident. In other words, parallel to the morphological differentiation of the species themselves there may be a morphological differentiation of their karyotypes. That the two sets of facts are causally connected in some degree is clear. *A priori* it is not to be expected, however, that the directly observable correspondence is necessarily close, for two main reasons. First, gene mutation unaccompanied by any structural changes in the chromosomes is alone capable of bringing about very extensive differentiation within a group of organisms. Secondly, on account of the small size of the chromosomes there are very definite limitations as to the amount of detail in their structure which can be made out with the microscope. (The Drosophilists recently may have hurdled this barrier.) This may mean that only the grosser features of the chromosomes pattern can be compared, the finer details, which may be immensely important, remaining entirely obscure. There is the further general consideration also that as development proceeds from egg to embryo, and from embryo to adult, there is a progressive unfolding of the inherited potentialities of the organism. Generally speaking, therefore, variations in adult form become increasingly difficult to establish the earlier the stage of development examined. In the germplasm their basis may not be visible at all. It follows that as a mode of delineating phylogenetic relationships, where groups are based on a large number of resemblances, comparative karyology, in its present stage of development, lacks the precision and the broad scope of comparative morphology. Nevertheless, it is proving to be a most helpful adjunct to the older phylogenetic criteria.

Of direct interest in the present connection is the extent to which the facts of comparative karyology shed light on the mechanism of evolution. The nature of the evidence which this approach to the problem affords is best illustrated by an example. It is proposed to con-

sider, therefore, some of the principal findings in the genus *Crepis*. I wish to acknowledge my indebtedness to Babcock and his associates on whose extensive and illuminating work in this field I have freely drawn.

THE GENUS CREPIS

The genus *Crepis*, of the family Compositae, comprises about 220 species widely distributed in the northern hemisphere and Africa. Approximately half of these species have been studied by the California workers. Three subgenera are recognized, *Catonia*, *Eucrepis* and *Barkhausia*, characterized in the order named by increasingly greater specialization of certain features of phylogenetic significance and, in general, by a reduction in length of life cycle and in size of plant and its parts. Supplementing the evidence from morphology and geographical distribution with extensive data on chromosome number and form, Babcock and Cameron (1934) attempt to work out the phylogeny of the genus. On the basis of the relationships indicated by these various criteria certain inferences are then drawn regarding the processes which appear to have been at work in the evolution of the group. Aside from the intrinsic value of the findings the study is important from the standpoint of methodology.

It is not proposed to marshall for presentation here the wealth of evidence which Babcock and his coworkers bring to bear on the problem. Rather I shall attempt merely to set out some of the more salient facts and then to indicate briefly the line of reasoning followed in interpreting the evolution of the group.

There is more than one basic chromosome number in each of the three subgenera. Common to all subgenera are the basic numbers 8 and 10. In *Catonia* and *Eucrepis* there is a third, namely, 12, and in *Eucrepis*, a fourth, namely 14. In the subgenus *Catonia*, species with basic chromosome numbers predominate, and this condition is associated with the persistence of primitive morpholog-

ical characteristics. In *Barkhausia,* the most highly specialized portion of the genus, likewise, all but three of the species studied have the basic numbers 8 or 10. The higher degree of specialization characteristic of this latter group is evidently the result of evolutionary processes which do not involve change in chromosome number. In contrast with the restricted variation in chromosome numbers found in *Catonia* and *Barkhausia,* the subgenus *Eucrepis* shows the following comparatively wide range: 6, 8, 10, 12, 14, 16, 40 and a series in multiples of 11, namely 22, 33, 44, and probably 55 and 88.

Further important evidence bearing on the phylogeny of the genus is afforded by a study of the comparative chromosome morphology. As first noted by Navashin (1925), there are three well-marked types of chromosomes in *Crepis,* distinguishable on the basis of the position of the constriction and by the presence or absence of a satellite. The first type, which has a subterminal constriction, is subdivided on the basis of total and relative length of the arms into three classes, A, B and C. In some cases there may be little difference between B and C. The second main type, known as D, shows a subterminal constriction and bears a satellite. The E chromosomes possess a small median constriction. All the 5-paired *Crepis* species have one pair each of chromosome types A, B, C, D and E. This is considered to be the basic genom. In the 4-paired species type E is lacking. In the two 3-paired species B or C, and E are not present. The 6-paired species are variable in their composition, each of the five chromosome types in the basic genom being reduplicated in one or another form. This fact suggests that 12 is not a basic number in *Crepis* and that these species are derived by hybridization from 3- or 4-paired species. In addition to the basic set, the 7-paired species show duplication of C, D or E. A hybrid origin for these species is likewise suggested. The 8-paired species, like the 4-paired species, have only

A, B, C and D types, and are evidently polyploids. The forms with higher chromosome numbers have not been analyzed in detail, but there appears to be clear evidence that the 11-paired American species owe their origin to inter-specific hybridization and amphidiploidy.

Without going into the further evidence which has been adduced on the phylogeny of *Crepis* it is evident that several evolutionary processes have been at work in the group. The most primitive species have 10 chromosomes, but there are likewise primitive 8-chromosome forms. Babcock and Cameron (1934) hold that the most fundamental evolutionary change in the genus is the mode by which the 8-chromosome species and, in turn, the 6-chromosome forms have been derived from 10-chromosome ancestors. Interspecific hybridization and amphidiploidy have also played an important rôle. All the species with 12 and 14 chromosomes are thought possibly to have been derived from amphidiploid hybrids.

Babcock and Cameron suggest that the reduction in chromosome number from 10 to 8 and from 8 to 6 may have come about through reciprocal translocation of a sort leading to the elimination of a pair of chromosomes. A change of this type has not been accomplished experimentally. Blakeslee, Bergner and Avery (1933) have succeeded in developing true breeding races of *Datura stramonium,* however, which possess 26, rather than the usual 24, chromosomes, and suggest a means by which a corresponding reduction in number might possibly be attained.

Changes in Chromosome Number in Datura

The starting point in the synthesis of the 13-paired races of Datura were stocks differing from the normal in chromosome structure and in possessing reduplicated chromosome segments. In one case, for example, the secondary called Sugarloaf, which possesses the reduplicated .2 end of the 1.2 chromosome, was crossed with Prime type 6, in which the .2 end is affixed to another chromosome, 11.12, while the 1. end remains as an inde-

pendently assorting fragment. Among the offspring of this hybrid one individual was found in which the normal 1.2 chromosome was lacking altogether, its place being taken by the two new units derived from this chromosome, namely, the 2.2 chromosome from Sugarloaf and the .1 fragment from Prime type 6, each in duplicate. The new race breeds true, although the 2.2 pair of chromosome behaves irregularly at meiosis, causing about 15 per cent. pollen abortion. In these Datura cases it is not known what relation the points of break in the modified chromosomes bear to the spindle fiber regions. If the .1 fragment embodies the spindle fiber region of the normal 1.2 chromosome, has the 2.2 chromosome acquired a new one? If, on the other hand, the spindle fiber region of the 1.2 chromosome is in the .2 end, then the 2.2 chromosome should have an extra one. The fate of the spindle fiber regions would appear to be a point of fundamental interest in connection with these true breeding forms with altered chromosome numbers.

To synthesize a Datura with one pair of chromosomes less than the normal, Blakeslee, Bergner and Avery point out that it would be necessary to transfer the two respective parts of one fragmented chromosome to two other chromosomes. Here again the question may be raised as to the disposition of the spindle fiber region of the eliminated chromosome. Aside from this obscure point, the results are suggestive of ways in which changes in chromosome number of the kind which Babcock and his associates consider fundamental in the evolution of Crepis may have come about.

Navashin (1932) emphasizes the fundamental importance of the fact that each chromosome has but one spindle fiber attachment, and that the number of chromosomes can not be increased or decreased without a corresponding change in the number of attachment regions. Fragments do not survive unless they retain a spindle fiber attachment or become joined to another chromosome or fragment possessing one. The problem of an

evolutionary increase or decrease in chromosome number may be looked upon, therefore, as primarily one of altering the number of spindle fiber attachments in the genom. Navashin suggests that changes in a plus direction may follow from the introduction in any one of the well-known ways of an extra chromosome which then becomes altered through "dislocation." Conversely, the chromosome number may be decreased through transfer of the materials in one or more chromosomes to other chromosomes, followed by the elimination of the spindle fiber region. As Navashin points out, if chromosome breakage is a random process changes of these kinds may be expected to occur from time to time.

McClintock (1932) mentions a case in maize in which a chromosome was broken in the spindle fiber attachment region with the result that both fragments received functional portions of it. Possibly this is a means by which chromosome number may be increased.

A fundamental difficulty which has long been realized in connection with the origin of new stable forms with chromosome numbers not multiples of the old numbers is the generally adverse effect on development occasioned by marked changes in gene balance. Quite aside from the meiotic irregularities resulting from reduplication or loss of parts of genoms, the effects on development of such changes in nuclear composition would seem gross enough, in the great majority of cases at least, to preclude the survival of the new type. The generally degressive character of gene mutations has given rise to the same dilemma, and we seem no nearer a satisfying solution in the one case than in the other.

It is to be borne in mind, however, that recognition of a cytogenetic process of possible evolutionary significance and obtaining a statistically adequate representation of its effects are two very different orders of things. If we consider only the possibilities inherent in duplication of chromosome segments of various sizes and in various combinations within a given species, for ex-

ample, the potential numbers are so vast as to be beyond easy comprehension. If the statistically almost negligible numbers of changes of this kind which have been subjected to laboratory study are found to be not advantageous to the organism we have no right, as has been pointed out by others, to argue that the process is not of evolutionary importance. While the great majority of alterations of this type may be disadvantageous, a particular few which are rarely realized in a system of random changes may have positive selective value. The only sound position to take appears to be this: While from a small sample of changes of a particular kind which have proved to be disadvantageous we can not deny the evolutionary importance of the phenomenon, we can not positively assert that the process in question is significant for evolution until one case at least is shown to be of this nature.

Structural Changes in the Chromosomes

Evidence has accumulated rapidly in recent years that structural changes in the chromosomes, while comparatively rare, are of wide-spread occurrence among plants. Alterations of apparently the same kinds as are found naturally may be induced by such agencies as x-rays, radium and heat. For purposes of the present discussion, these structural changes may be grouped into two general classes on the basis of their effects on the organism. There are, first, deficiencies and duplications which (if not compensated for) occasion a change in the gene balance and, secondly, inversions and translocations which do not result necessarily in any alteration in the genom other than in the position of certain of its components.

Usually, at least, deficiencies in homozygous conditions are lethal and therefore would appear to be rarely, if ever, of evolutionary significance. Duplication of chromosome parts, on the other hand, may be an important source of evolutionary differentiation. Their effects

on development must vary widely, depending on the gene content of the reduplicated part, but observation shows that they are very often compatible with survival. If the reduplicated segment is a fragment attached to another chromosome the essential basis is provided for regular behavior in somatic cell division and meiosis. The field is a relatively new one and there is little definite evidence for the rôle which duplications have actually played in the formation of species. It is a reasonable postulate, however, that a part of the visible differences in the genoms of related forms are attributable to this type of structural alteration. Duplication might likewise be expected to give rise to character changes through the establishment of new modes with reference to gene balance.

The rôle which inversions have played likewise awaits the results of further study. Emerson and Beadle (1932) have shown that, while crossing over in hybrids between maize and Florida teosinte occurs with approximately the same frequency as in maize in parts of four chromosomes tested, it is markedly reduced in a segment of the *C-wx* chromosome. It is suggested that the teosinte segment in question may be inverted relative to its homolog in maize.

RECIPROCAL TRANSLOCATION

A striking type of nuclear change which appears to have evolutionary significance is the exchange of segments between non-homologous members of a set. The process, first recognized by Belling, is termed reciprocal translocation. It is detectable cytologically by the occurrence of rings or chains at the first meiotic metaphase in plants heterozygous for the structural peculiarity. Originally postulated to account for the aberrant behavior of certain Daturas, it has since been definitely established in various other flowering plants and is probably of rather wide-spread occurrence. This type of chromosome alteration does not alter the gene balance.

Several reciprocal translocations of spontaneous origin have been recognized in maize, in which the phenomenon is readily discovered by its characteristic effect in reducing the number of seeds per ear to about one half of normal.

Strains of maize homozygous for reciprocal translocations are fully fertile and breed true for the new chromosome arrangements. Chromosome 1, the longest member of the complement, is known to be involved in at least three different translocations which have arisen spontaneously. In these cases the breaks have occurred in different places in the chromosome and it appears not unlikely that the process of breakage is a random one. Hybrids between the translocated races and normal maize are partially fertile, the degree of sterility depending upon the number of translocations involved and their relations to each other; and to some extent upon the position of the breaks. From stocks now in hand it is theoretically possible to synthesize fully fertile lines which, in crosses with ordinary maize, would give entirely sterile hybrids. It is evident, therefore, that reciprocal translocation is a potentially effective means of physiologically isolating races within an otherwise freely interbreeding group.

There is as yet little exact evidence bearing on the question of possible character changes accompanying reciprocal translocations in maize. As mentioned above, the new structural types are readily obtainable in homozygous condition and breed true. None have been observed to show any conspicuous changes in morphology. The only attempt which appears to have been made to obtain a closer comparison of a new structural type with normal maize of the same genic composition is one which the writer carried out a few years ago (Brink, 1932). The numbers of individuals involved were rather small, and no statistically significant difference was found between the two types in total weight of plants, weight of ears at maturity or in rate of development as measured by number of days to emergence of the silk. While the

available data on the question are small there is no positive evidence in maize, therefore, for a phenotypic position effect accompanying translocation.

Conspicuous success in accounting for the peculiar constitution and breeding behavior of Oenothera has attended the application of the concept of reciprocal translocation to that group (Darlington, 1929; Blakeslee and Cleland, 1930). The persistence of structural hybridity as a regular feature of certain Oenotheras, however, is apparently not a consequence of the translocations alone, but is dependent on an associated complex of lethal factors. The comparative rarity of structural hybridity among plants and the fact that in the species in which it occurs it is maintained only at the cost of considerable sterility, would appear to minimize the evolutionary significance of reciprocal translocation in this relation.

As a factor in the differentiation of species, however, reciprocal translocation appears to have played a genuine part. In *Datura stramonium,* Blakeslee (1929) has shown that different geographical races vary in the structural make-up of their chromosomes. In the United States and possibly in Brazil, a form known as the standard Line 1 is the most common. In other countries, however, the B-type, which differs from Line 1 with reference to the position of certain segments in two non-homologous chromosomes predominates. Races from Peru and purple-flowered forms from Chile give evidence that they differ from the standard Line 1, not only as does the B-type, but also in two other pairs of chromosomes. Thus there is evidence of a differentiation with respect to chromosome structure of possible evolutionary significance within limits of this one species. Bergner and Blakeslee (1932) have tested one inbred line each of two other species, *D. quercifolia* and *D. ferox.* Both these lines proved to be B-types, conforming in this respect to the most widely distributed *stramonium* form. Both *ferox* and *quercifolia* were found to differ from B-type *stramonium,* however, with respect to two other pairs of

chromosomes. If the ends of these chromosomes in *stramonium* are represented in Blakeslee's terminology as 11.12 and 21.22, then the other two species are 11.21 and 12.22. Evidence was likewise obtained that chromosomes 7.8 and 19.20 in *stramonium* are replaced by 7.20 and 19.8 in *quercifolia*. In the development of *ferox*, a further interchange involving apparently only the "hump" on two pairs of chromosomes in *quercifolia* has occurred. While, as pointed out above, these relations are based upon analysis of only one line each of *quercifolia* and *ferox*, they are suggestive of the conclusion that in the evolution of Datura reciprocal translocation resulting in changes in chromosome morphology has played a part.

At the present meetings Bergner and Blakeslee present further evidence on this point. It is shown that *Datura discolor* in hybrids with *D. stramonium* forms a ring of 10 chromosomes plus seven bivalents. *D. ferox* × *D. discolor* gives a ring of 10, or a ring of six with an attached ring of four, and seven bivalents, in one of which the chromosomes are heteromorphic. *D. quercifolia* × *D. discolor* forms two rings, one of six and one of four chromosomes, plus seven bivalents one of which again consists of unequal members. These facts added to those earlier established point to the conclusion that in the genus Datura reciprocal translocation may have played a fundamental evolutionary rôle.

In *Zea mays*, on the other hand, structural changes in the chromosomes do not appear to have played a part in the differentiation of the extremely wide range of forms within the species. Dr. D. C. Cooper and I have recently been looking into this question. Through the kindness of Mr. J. H. Kempton, U. S. Department of Agriculture, who put the seed at our disposal, we have been able to examine a series of corns now being grown on Indian reservations in this country. These strains are mostly either flinty or floury and, generally speaking, are much smaller in size than the commercial varieties of the Middle West. The method used to detect structural differ-

ences was to cross each strain with our standard line, and to examine the pollen of the hybrid. If significant amounts of aborted pollen were not found it was assumed that there was no difference, in gross chromosome structure at least, between the parent forms. The 17 United States Indian corns tested in this way all proved to be normal.

Dr. L. F. Randolph has recently been making a survey of chromosome morphology in maize. The results are unpublished, but Dr. Randolph kindly permits me to mention that certain strains from southwestern United States possess chromosomes more nearly like those of Euchlaena than any previously known. In our own studies corns from this region were not included.

A more extensive examination has been made in our laboratory on a representative series of Central and South American corns. The Central American corns were collected for us by Professor H. H. Bartlett, University of Michigan, and Mr. O. G. Ricketson, Carnegie Institution of Washington, in charge of the Uaxactun project in Guatemala. The South American material was assembled by the late Professor Erwin Baur and sent to us by Mr. F. D. Richey, U. S. Department of Agriculture, with whom the collection was shared. We wish to take this opportunity of acknowledging our great indebtedness to these cooperators.

The range of variability in type among these corns is enormous. One might contrast, for example, a very small seeded form with a distinct beak, from Ambotos, Ecuador, similar to some of our varieties of pop corn, with the giant-seeded flour corns from Cuzco, Peru, weighing more than ten times as much. Similarly the flint corns being grown at altitudes of approximately 8,000 feet in Guatemala are very different from the types being cultivated at sea level in the same region. Yet the chromosomes in these widely different races of maize appear to be structurally the same.

The plan followed in testing this material was to cross each strain with one or more lines carrying transloca-

tions involving known chromosomes. The hybrids were then examined cytologically, the configurations at meiotic metaphase I being noted particularly. If the chromosome rings expected on the basis of the reciprocal translocations introduced were found, and pairing among the other chromosomes was regular, it was concluded that the chromosomes of the race under test were structurally normal. There were examined in this way one strain from Mexico, 19 from Guatemala, 13 from Peru, 13 from Ecuador, eight from Bolivia and one from Chile. Only one hybrid was found which showed an abnormal configuration. It was a cross between a stock from Riobamba, Ecuador, and a tester strain carrying the translocation, T1-7. On the basis of normality, a ring of four chromosomes and eight bivalents was expected, whereas a ring of six chromosomes and seven bivalents was found. Only a single plant from this lot was tested. Eight other plants from as many different lots from the same region, however, behaved normally. It is probable, therefore, that the structural condition inducing a ring of six, rather than of four, chromosomes was peculiar to the particular plant used in the cross. The evidence is clear, at least, that the difference found is not typical for the corns of that locality.

Between Zea and Euchlaena, on the other hand, there is evidence of structural differences in the genoms. Morphologically these two genera are widely unlike, but they may be readily crossed and give rather highly fertile hybrids. Beadle (1932) found that the relative lengths of the ten teosinte chromosomes were approximately the same as those of maize, although there are differences in the positions of the spindle fiber attachments in two members of the complement. Moreover, knobs, which are rarely found on maize chromosomes, are regularly observed at one or both ends of the teosinte chromosomes, except on numbers 5 and 9. Of the three varieties of *Euchlaena mexicana* which have been studied in hybrids with maize, Chalco and Durango show fairly regular meiotic behavior. Furthermore, Emerson and Beadle

(1932) have shown that in the intergeneric hybrids crossing over occurs with approximately the same frequency as in maize in parts of eight chromosomes in the case of Durango and in parts of five with Chalco. In maize × Florida teosinte hybrids, however, there are frequently two unpaired chromosomes during diakinesis and metaphase. This fact, observed by Kuwada and by Beadle (1932), is confirmed by Arnason (unpublished) working in our laboratory. By using various maize translocations as markers, Arnason has adduced evidence that one of the heteromorphic chromosomes may be number 5 and that the other is probably number 8, 9 or 10. Beadle and Emerson (1932) found that the frequency of crossing over in maize × Florida teosinte hybrids in a region corresponding to 52 map units in the ninth maize chromosome is very strongly reduced. The hybrid with Durango teosinte behaves similarly. Beadle associated this reduction in crossing over with atypical behavior of the ninth chromosome, an observation which Arnason's work suggests is probably correct. The intimate nature of the structural difference in the chromosome is still obscure.

Reciprocal translocations are known to occur spontaneously in maize, and reveal their presence by the induction of chromosome rings at meiotic metaphase I. It is possibly a significant fact that ring formations involving more than two chromosomes do not occur in maize × teosinte hybrids. The lack of direct evidence for reciprocal translocation raises the question whether the phenomenon has played a rôle in the evolutionary differentiation of this group of plants. There are structural differences in the genoms of maize and teosinte, but they do not appear on the basis of the present evidence to be of this kind.

Darwin's conception of the part played by natural selection in the evolution of species stands to-day much as he built it, a pillar whose foundations subsequent biological research has not shaken but whose essential lines are being steadily brought into clearer relief. Even as Darwin perceived it, however, the eventual solution of the

problem of evolution is not to be represented as a single column, but rather as an arch whose two respective piers, symbolizing the selective forces of the environment on the one hand and genetic variations on the other, are united in one structural unit. Whence come the genetic variations? Some of Darwin's ideas in this respect have not stood the test of time. The beginnings he made in building that pillar mostly have been torn down. We are now engaged in an attempt to build it anew. How much progress has been made? We are sure at least that not all the blocks out of which this pier are built are of the same class, and it would seem that they come in random sizes. Perhaps we have not recognized all the different kinds, but we believe that some of them have been found. For over three decades geneticists have been quarrying for these blocks. If they now feel justified in occasionally venturing out to try their skill at putting them together it is a good sign.

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ROLE OF GENES IN EVOLUTION[1]

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EVOLUTION is not always a slow process, sometimes it is a speedy one. The evolution of ideas on heredity brought about through researches in genetics which began a little more than three decades ago was very rapid. During that short period of time vague ideas about discontinuous variations among organisms and theories based purely upon speculations evolved into the present science of genetics. Progress was rapid from the start. Mendel's laws established the fact that heritable characteristics are transmitted from one generation to another through definite bodies which were later named genes. Soon after that genes were connected with chromosomes. It was found that genes have a definite position within a chromosome, that their order is linear and that an interchange of genes between homologous chromosomes may take place through crossing-over. It was also discovered that a gene may assume several different forms called allels. All the work up to that time and later work which followed showed that the nucleus is almost entirely responsible for the transmission of hereditary characteristics. The extra nuclear material, though essential as an interacting agent with the nucleus for the development of an organism, was found to be responsible for the transmission of a very few, if any, of the hereditary characteristics. The discovery of unstable genes (Emerson, 1914) which made possible the quantitative measurements of certain gene changes contributed materially toward the analysis of the gene problem. Genetic research, also, gave a definite answer to the question which for a long time has been a center of controversy, *viz.*, the question of the influence of environment on heredity. It is now clear that the

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environment in which an organism lives does not induce hereditary changes. Such changes do occur every once in a while, but their occurrence is a random rather than a directed process. The environment, however, does act as a selective agent by preserving from among the gene changes which are continuously occurring those which are favorable under the given conditions. Muller's (1927) discovery that x-rays produce changes in genes marked an important forward step in genetic research. It gave to geneticists a long-desired tool to hasten still further the progress of genetic research. The recent discovery that the structure of the salivary gland chromosomes of Drosophila is correlated with the genetic loci (Painter, 1933) is giving another impetus to genetics. X-rays and the salivary chromosome studies made it possible to attempt an experimental solution of problems which were but a mere speculation a few years ago.

Genetics has made such fast progress that many biologists were overtaken by it; some preferred to disregard genetic findings, rather than to abandon their pet ideas; others could not accept these new facts because they seemed too revolutionary. To-day there is still talk in certain quarters of the inheritance of "acquired characters," although ample genetic evidence has now been accumulated for a satisfactory solution of this problem. This presentation is not intended to be a sermon to nonbelievers; rather it is an elaboration of a working hypothesis summarizing my interpretation of a phase of genetic research.

Before discussing the rôle of the gene in evolution it is essential to say a few things about the gene itself. I shall begin, therefore, with a presentation of a gene concept or a working hypothesis of the nature of the gene.

A Working Hypothesis of the Nature of the Gene

Undoubtedly, the most important property of the gene is its power to reproduce. This is also one of the main characteristics of living matter. All evidence now avail-

able indicates that a gene reproduces exactly. A wild-type allel of white locus, for example, reproduces as a wild-type form, but if it should change into some other allel, let us say eosin, it will reproduce this new form until some other change in the gene occurs. As will be shown later, it is reasonable to assume that changes which differentiate one allel from another are slight changes probably due either to molecular rearrangements in the gene molecule or to slight changes within molecular groups of that molecule. Yet the new forms embodying these changes reproduce exactly thousands and thousands of times. How this happens and what mechanism is responsible for this reproduction puzzles the biologist as much as it puzzles a chemist and a physicist. An answer to these questions would be a long step toward the understanding of the basic processes of life. Since a gene appears to be the smallest and probably the simplest unit possessing self-reproducing property, this answer may come from studies of the physico-chemical properties of the gene. At present, however, possibilities for such studies still seem to be distant.

Estimates of the size of the Drosophila genes made by several geneticists (Morgan, 1922; Muller, 1929; Gowen and Gay, 1933) indicate that a gene is a very minute particle. These estimates were arrived at by computing the volume of the chromosomes and dividing it by the computed total number of genes. The obtained values give the upper limit for the size of the gene. The lowest value, reached by Gowen and Gay, places the diameter of the gene at 10 millimicrons, Muller's estimate at 50 millimicrons and Morgan's at 20, 60 and 70 millimicrons. In these estimates in computing the volume of chromosomes the chromatin material was included. Since there is good reason to believe that this material is non-genic, values obtained for the size of the gene are much too high. It seems very probable that the volume of the extragenic material of metaphase chromosomes is many times as large as the volume of the genes themselves. If this is

taken into account, then, the estimate of the size of a gene is brought down into the range of the size of a large organic molecule.

The known properties of the gene, such as self-reproduction, allelism, the behavior of unstable genes and changes produced by x-rays, are much more easily explained on the assumption that genes are single molecules rather than aggregates of molecules. Our present knowledge of the structure of the gene, however, is far too meager to allow us to build up anything but a working hypothesis concerning its nature. Since the single molecule assumption appears at present the more probable of the two, it will be used in the working hypothesis to be developed in this paper. A gene, therefore, is visualized as a single organic molecule and changes in the gene as due to either rearrangements or changes in molecular groups constituting that molecule.

Disregarding changes occurring in unstable genes, which will be considered later, two types of changes are evident in genes, *viz.*, the visible and the lethal ones. Either of them may occur in the same locus. Both types of changes are found under natural conditions and both types also appear as a result of x-ray treatment. Since in Drosophila the proportion of these two changes is similar in the treated and in the non-treated material (Patterson and Muller, 1930) the conclusion seems justified that in both cases a similar mechanism is responsible for their origin. A large number of these changes have been obtained in the x-rayed material, and their analysis is therefore feasible.

In experiments which I have been conducting for almost two years a great many changes have been observed in certain loci of the X-chromosome. In Table 1 the data are summarized for loci for which the fertility was good. It is evident from these data that out of 49 fertile changes observed, 34 (or 69 per cent.) were lethal and 15 (or 31 per cent.) were viable changes. Similar data were reported by Patterson (1932a), showing that out of 128

fertile changes observed by him in 14 loci, 112 (or 87 per cent.) were lethal and 16 (or 13 per cent.) were visible changes.

TABLE 1

CHANGES OBSERVED IN CERTAIN LOCI IN THE X-RAYED MATERIAL

	Total	Fertile	Lethal	Viable	Per cent. lethals
w	11	11	7	4	
ct	16	16	14	2	
dy	6	5	5	–	
g	4	4	3	1	
f	14	13	5	8	
		49	34	15	69.4

In all loci which I have investigated 62 lethal changes were observed. For 53 of these genetic tests have been completed which indicate that they are deficiencies. As a criterion for deficiencies, the following characteristics were used: lethal effect, cell-lethal effect (36 cases), the exaggeration effect for certain loci, and in a few cases, the fact that more than one locus was affected by the change. For 18 of these lethals the study of the salivary gland chromosomes confirmed the conclusion that they are really deficiencies. This evidence makes it probable that all or at least the great majority of these lethals are deficiencies.

The genetic length of these deficiencies can best be seen from the analysis of a series of 56, which include also non-lethal bar deficiencies. Tests with known adjacent loci showed that 37 included one known locus only, 17 included two loci, 1 three loci and 1 four known loci. The longest one, including *w–fa–ec* loci, has the genetic length of four units. The cytological studies of salivary chromosomes show, however, that the cross-over values are not always a good measure of physical distances between the genes. The *w–fa–ec* deficiency, for example, includes about four bands, and it is not any longer cytologically than a *f–B* deficiency which genetically includes a section

0.3 units in length. Bands for *pn*, *w* and *fa* appear adjacent, but in spite of this the genetic length of the *pn–fa* section is 2.2 units. On the other hand, the section of the chromosome to the left of *pn*, which is genetically only 0.8 units long, contains about 50 bands. On 18 deficiencies cytologic analysis of salivary gland chromosomes was made, and it has been found that 7 were deficient for one band only, 3 for two bands, 3 for three, 2 for four and one each for five, six and seven bands. All this indicates that the most common deficiencies involve only a minute region of the chromosome.

The question now arises as to how these deficiencies originated. It seems to me they may have been formed either by the mechanical process of a piece of the chromosome being pinched off or through a chemical change which eliminated a section of the chromosome. Whatever their origin is, however, they seem to form one series with a peak frequency at a single locus, suggesting that all of them originated through a similar process. Since none of the deficiencies considered here is terminal, none of them could have originated through a loss of a small end piece of the chromosome, and since the peak frequency is at the single locus, it is unlikely that they could have been produced by a pinching-off process. It is very probable, however, that some deficiencies originate through the physical loss of a section of a chromosome. As suggested by Dubinin (see Muller, 1932), where the different regions of the threads of the same chromosome cross each other they may stick or fuse together. If, then, when the chromosome straightens out, this fusion remains and the looped section is dropped out, such a process would be responsible for a deficiency. It would be expected, however, that deficiencies originated in this manner would include a rather large section of a chromosome. They could properly be called deletions, a name applied by Muller (1932) to all deficiencies. It seems very probable that the deficiencies analyzed here do not overlap with deletions and also that deletions are very much less frequent than deficiencies. Unequal crossing-

over offers another possibility for the origin of deficiencies through a mechanical process (Sturtevant, 1925). In the material considered here, however, all deficiencies were induced in males, where it is known that crossing-over occurs very rarely so that this possibility needs not be considered.

It seems to be probable, therefore, that small deficiencies, which are the most common ones, originate through chemical processes of changes in genes. How these changes are brought about in the x-rayed material is not important in this discussion. They may be either a result of a direct action, an electron hit producing chemical changes in a gene directly or they may be caused indirectly, an electron hit producing a change in the environment of a gene which in turn affects the gene itself. In case of a direct hit, the adjacent genes would be affected through a chain reaction stimulated by a primary change produced in a gene. Data, which are slowly accumulating in my experiments, suggest that the direct action is the more probable of the two. So far I have obtained three viable deficiencies for the miniature locus; and two more have been reported by Patterson (1932a) and genetically tested by him. All these five deficiencies affected miniature locus only; none included the adjacent locus dusky. On the other hand, every one of the six deficiencies detected up until now for the locus dusky included miniature also. If a change in a gene were produced through the effect of the gene environment it would be difficult to visualize why a change in the dusky locus was always accompanied by a change in the miniature and a change in the miniature did not affect the dusky. More extensive data of a similar nature may make it possible to answer the question of how changes in genes are produced by x-rays.

A group of genes is known in which changes occur with a high frequency. These are the so-called unstable genes. The type of the changes in these genes indicates that a definite process is being repeated at a relatively high rate. In all known unstable genes changes, at least

the most frequent ones, always go from one allel into the other allel. For example, the unstable gene for rose Delphinium changes into the purple allel, for white pericarp of maize into the red allel, for miniature wings of *Drosophila virilis* into the wild-type allel. This regularity in the change suggests that the process responsible for it may be a reversible chemical reaction. Unstable miniature originated from wild-type; and it reverts back to wild-type. A logical explanation of this occurrence seems to be that the chemical change which produced the original unstable miniature is a reversible reaction; reversions occurring with a relatively high frequency. This conclusion is supported by the finding that the rate of reversions is influenced by various internal factors such as stage of ontogenetic development, sex, and also by genetic factors (Demerec, 1929, 1931, 1932b). Even these unstable genes, however, were found to be beyond the reach of the influence of external factors such as high and low temperature, carbon dioxide and x-rays: none of these factors affect unstable genes to any appreciable degree (Demerec, 1932a, 1934a).

Disregarding changes occurring in unstable genes, since they are probably of a different order than the changes in other genes, it is evident that the great majority of the detectable changes are deficiencies. That does not necessarily mean, however, that the majority of all changes occurring in genes are such. Undoubtedly there are many viable changes which can not be detected, since they either do not affect the organism or affect it slightly. Now comes the question of how these deficiencies are produced. To produce a gene deficiency it is not necessary to destroy the gene. It is sufficient to affect it in such a way as to make it lose its power of reproduction. If that happens, the gene is automatically eliminated at the next cell division. This seems to be the most probable origin of deficiencies. Since the power to reproduce is a highly specialized property it is not surprising that it is easily lost and that a large proportion of gene changes belong to the group of deficiencies.

As far as it is known at present the deficiency for the bar locus is the only one among about 30 deficiencies known in *Drosophila melanogaster* which is not lethal to the organism when in homozygous condition. This indicates that the presence of the great majority of loci is essential in order that an organism may live. It has been shown in another paper (Demerec, 1934b) that even a cell without a full complement of the majority of loci can not function properly. Out of the 14 regions of the X-chromosome tested only one has been found not showing the cell-lethal effect. That indicates (Demerec, 1934c) that probably every gene performs a function in every cell and that the presence of a majority of them is essential in order that a cell may function properly.

To summarize, a gene is a minute organic particle, probably a single large molecule, possessing the power of reproduction, which power is one of the main characteristics of the living matter. Changes in genes (mutations) are visualized as changes or rearrangements within molecular groups of a gene molecule. It looks as if a gene can stand slight changes only; a more extensive change destroys its reproductive power and automatically eliminates the gene. A whole complement of genes of an organism constitutes a balanced system which determines the appearance and the nature of that organism. A majority of genes of a gene complex probably functions in every cell. If any one of them is missing the disturbance produced in the system is lethal to the whole organism and in many cases is even highly detrimental to individual cells.

Gene as an Evolutionary Factor

The following discussion is based on the genetic concept which is prevalent among geneticists interested in this subject. No claim is made here for the originality of the ideas expressed. Most of them are logical conclusions and in some cases the only logical conclusions which could be drawn from the evidence accumulated through experiments of a large group of geneticists.

As already mentioned earlier, the whole complement of genes of an organism forms a system which interacts with the environment to make up the phenotype. This system seems to be balanced and adjusted to the environment in which the organism lives. A change in the environment as well as a change in the gene system disturbs the balance and produces an effect which is liable to be detrimental to the organism. This has been amply demonstrated by a large number of visible and lethal mutations which occurred under experimental conditions in Drosophila and all of which are detrimental. As pointed out earlier, in addition to detectable mutations, there are undoubtedly some which affect the organism so slightly that they can not be detected. It seems reasonable to assume that such mutations may be fairly frequent, probably at least as frequent as lethal changes and also that they affect the balance of the gene system to a lesser degree than either lethal or visible changes. The degree of effect is probably a graduated one, varying from a very slight one and continuing up the scale until the visible effect is reached.

In the gene system, therefore, there are constantly occurring three types of gene changes which are similar in nature but differ from each other in the degree of their effect. These changes are the lethal changes due probably in the majority of cases to gene eliminations, visible changes due to chemical changes in genes which have a strong effect on the organism and invisible changes due to chemical changes which have a slight effect on the organisms.

What is the evolutionary significance of each type of change?

It is evident without much elaboration that the lethal changes and especially those produced through gene deficiencies can not have any evolutionary significance. All these changes are highly injurious to the organism; a majority of them are probably cell-lethal so that they are rapidly eliminated from the gene complex.

The second type of gene changes, *i.e.*, the visible muta-

tions, because of their detrimental effect do not offer an effective mechanism for the evolutionary progress except in circumstances where due to a change in the environment a particular mutation either loses its detrimental effect or becomes beneficial. The thermal strain of Daphnia described by Banta and Wood (1928) offers a good opportunity for such an occurrence. Normal Daphnia lives in water which has a temperature range of 12 to 26 degrees Centigrade, the optimum being 17 to 21 degrees. Apparently due to a mutation a thermal line originated having a temperature range of 21 to 32 degrees and the optimum of 25 to 28 degrees Centigrade. In Daphnia, therefore, mutations of a certain locus affect the range of temperature at which the organism is able to live. Dr. Banta estimated that a relatively small Daphnia pond of 50 x 50 x 0.5 meters contains at the peak of the season about 2,500,000,000 developing embryos. It is reasonable to assume that the mutations in the thermal locus occur with the frequency of average loci, and if so it may be expected that among a population of $2\frac{1}{2}$ billion embryos there would be at least one homozygous for the high temperature allel. If, therefore, any change in the climatic conditions should occur during the peak of the Daphnia season which would increase the temperature of water to about 28 degrees such a condition would be lethal to the normal Daphnias, but it would be advantageous to the thermal individuals. If the high temperature of the water should persist the result would be the origin of a thermal strain and the establishment of an effective barrier between the normal and the new strain. In this and in similar cases a detrimental change in the gene may become beneficial through a change in the environment and may play a rôle in the evolutionary processes.

The third type of gene change, *viz.*, those having a slight effect on the organism, play, probably, a more important rôle in the evolutionary processes than the changes just mentioned. Since the unbalance in the gene system produced by such mutations is slight, the selection

against their persistence is slight also. Moreover, the natural variability in the environment makes some of these changes neutral as far as the balance of the gene system is concerned. Such mutations, therefore, may account for the slight differences occurring between various strains and if allowed to accumulate through some barrier which produces isolation these differences may become great enough to be classified as different species. The lines differentiated in this manner, however, would be expected to cross readily and to produce hybrids, since the difference between any two members of the allelomorphic pair would not be great enough to produce such an unbalance in the system as to cause sterility. Sterility in crosses would arise if any of the loci becomes so differentiated that it does not resemble the original locus. That could happen through repeated mutations in the same locus which could persist because of changes in the requirements for the balance of the gene system due to mutations occurring in various other loci. The persistence of loci, such as white, yellow, scute, miniature, etc., not only within different lines of the same species but also in widely separated species, such as *Drosophila melanogaster* and *D. virilis,* indicate that at least the loci playing an important rôle in the balance of the gene system have a limited range of changes, any change which would significantly affect the locus disturbs that balance to such an extent that the new form is eliminated because of its detrimental effect on the organism.

Sterility in crosses is one of the most important means for accumulation of differences and thus for the formation of new species. Since by the addition of new loci to a gene system sterility may be more readily produced than through differentiation of genes it seems to me that addition of loci is a more effective means for the evolutionary progress than changes in genes. As pointed out in an earlier paper (Demerec, 1933), if a whole complement of genes is necessary for an organism in order that it might live or even for a cell in order that it might func-

tion properly then the fact that one line possesses only a few loci not present in another line would produce a high degree of sterility among the offspring produced by crossing these two lines. "Even a difference in one locus of the type described by Patterson (1932b) as a viability gene would be sufficient to produce an almost complete incompatibility between two lines, one possessing the gene and the other not possessing it." Such sterility, then, would permit the accumulation of genic differences within the group isolated through it.

It is hardly probable that genes originate *de novo*. A gene is, probably, the ultimate unit of life and as such could come into existence on extremely rare occasions. The addition of new loci to a gene system therefore must be accomplished through the duplication of the old ones. A few of them could be added to a gene system through various chromosomal abnormalities such as unequal crossing-over, duplication and translocation and a full set of them could be added through tetraploidy or duplication of the type observed in certain interspecific crosses. The experimental evidence indicates that whenever less than a full set of genes is added an unbalance in the gene system results which is detrimental to the organism. This unbalanced condition appears to be roughly proportional to the number of duplicated loci. Some of the single or a few loci duplications, therefore, may be expected to produce a slight unbalance similar to that produced by gene changes having a slight effect. Such duplications would not be very injurious to the organism and would not be readily eliminated. Duplicated loci, however, would have more freedom for changes than the other loci, since their activity would not be essential to the organism. Soon, therefore, they would differentiate from the original form and become new loci. In time the gene system would get adjusted to new loci, they would become established as an integral part of that system and as such become essential to the organism and to the cell. This process would bring both differentiation and steril-

ity in crosses to the group of the organism in which it occurred and would be a long step toward the formation of a new species. That the addition of loci is an important factor in the origin of new species is indicated by the fact that all new species formed experimentally up to the present originated through that process.

In concluding this presentation it may be stated that all genetic evidence accumulated so far indicates that the gene offers an efficient mechanism for the evolutionary progress of living organism. Just how this progress is accomplished is not known, but genetics may be soon in the position to offer a more definite evidence on this subject than is available at present.

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FACTORS OF EVOLUTION IN FOSSIL SERIES[1]

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INTRODUCTION

FOSSILS are remains, impressions or traces of organisms of the geologic past, preserved in the rocks. They therefore form our only direct, contemporary records of evolution through all but a minute fraction of the known history of life.

These records admittedly are incomplete. Not that fossils themselves are few: uncounted thousands are preserved in museums, while millions lie in strata at or near the surface, where they are destroyed by weathering and erosion far more rapidly than they can be collected. Nor are the series among them grossly incomplete: they compare favorably with any that may be constructed from living species. Their number, perhaps, is not great—but experience has shown that it may be increased by the simple process of taking specimens from taxonomic sets and arranging them in stratigraphic sequence. Need for and comparative simplicity of taxonomic work merely have not given them emphasis.

The really essential gaps, replacing those stressed by Darwin in his oft-quoted chapter "On the Imperfection of the Geological Record" (Darwin, 1872, pt. 2, 54–95), lie within the fossils themselves. Much can be buried and much preserved: witness birds with feathers, ichthyosaurs and cephalopods with outlines of flesh, and the remarkable holothurians, annelids and algae of the Burgess formation, of Middle Cambrian age. Generally, however, each process involves great loss, so that from trees there remain isolated leaves, from annelids a few detached parts, from snails or brachiopods only shells. Even when traces of flesh are preserved, they are useless for either experiment or cytologic investigation, rarely

[1] Presented at the symposium of the American Society of Naturalists at Pittsburgh, December 29, 1934.

being more than films of carbon. Any applications of genetics, cytology or physiology to fossils therefore must be made by way of morphology, with analogies as their principal basis.

When those applications deal with evolution, however, the paleontologist has certain advantages. His series have far greater scope than any dealt with by neontologists, involving time ranges of thousands, millions or hundreds of millions of years. He need not speculate on what will or will not survive: most if not all his material is extinct, and the order of its disappearance is a measure of its ability or inability to exist. Conversely, the order of their appearance indicates with considerable precision the order in which new types arose, while their frequency in collections which are essentially random samples indicates, through long periods of time, the frequency of mutations and other heritable changes. Finally, from series both restricted and comprehensive, with origins and endings established, the paleontologist is able to deal with the perennially vexed question of determinacy or indeterminacy in heritable variation: with orthogenesis and heterogenesis.

This statement of advantages and disadvantages outlines the scope and objects of this paper. It attempts to summarize the essential characters of fossil evolutionary series, applying to them generalizations derived from genetics and cytogenetics. It then discusses one special type of series, degenerative and orthogenetic, which has been productive of much confusion in the past, yet which seems exceptionally fitted to genetic and physiologic interpretation. It does not, however, present any interpretation as final, nor as general for all or even a majority of evolutionary series.

NATURE OF PALEONTOLOGIC SERIES

Many discussions, whether heterogenetic or orthogenetic, Buffonian or Darwinian, seems to assume that all paleontologic evolutionary series are of one type. In-

stead we may distinguish three, which differ in taxonomic scope, duration, morphologic results and (apparently) in cytogenetic and external processes involved. Adopting, without philosophical or ethical implications, terms that characterize their outstanding morphologic features we may term them progressive, stable and degenerative series.

Progressive series are those which many evolutionists term "emergent" and which Hurst (Hurst, 1932) has called "creative." They include families, orders or even phyla; they extend through ages amounting to millions or hundreds of millions of years. They involve the production of conspicuously or radically new types with new or remarkably specialized structures and functions which enable them to thrive in new environmental complexes. These new types either are able to survive for considerable epochs, or produce descendants with that ability. Familiar examples are trilobites, therapsid reptiles, birds, camels, horses and proboscideans, all discussed in standard text-books on evolution.

Stable series range from Linnean species to phyla, with corresponding variations in the time encompassed by them. They involve the production of no radically new types, though species, subspecies and variants not taxonomically distinguished are abundant. There is little or no adoption of new environments, though several groups falling into this class have taken refuge in fresh water as the seas became more salty (Twitchell, 1929, 1934; Pearse, 1927). With this conservatism is combined great geologic range, with or without marked diminution. Examples are:

Lyssacina (Hexactinellida)—Mid-Cambrian to Recent, 500,000,000 years.
Monactinellida—Mid-Devonian to Recent, 325,000,000 years.
Lingula (Brachiopoda)—Ordovician to Recent, 440,000,000 years.
Atrypa "reticularis Linn." (Brachiopoda)—Silurian and Devonian, 60,000,000 years.
Limulus (Xiphosura)—Triassic to Recent, 200,000,000 years.
Phoronida—Early Cambrian to Recent, 540,000,000 years.

Degenerative series generally are more restricted than either of the others, commonly occurring within families, genera or even species. Like stable series, they involve the production of no radically new forms and the adoption of no new environments; unlike them, speciation is manifested primarily in diminution or loss of early structures and abilities. This is paralleled by decline and extinction within epochs geologically and even historically short. Like the elaborations encountered in some trilobites, ammonoids, ceratopsian dinosaurs and titanotheres, degeneration commonly may be accepted as an inverse measure of further racial existence.

This relationship is not invariable. Some organisms appear to have undergone degeneration to a moderate degree and then—perhaps under the influence of sheltering environments—to have become stable. Others, especially parasites, have carried degeneration to extremes, yet seem to prosper and even increase. In many of them, however, degeneration has been accompanied by specialization of functions or organs so radical as to link them with progressive series: even *Sacculina* is more than a degenerate barnacle. Parasites and degeneratives comparable to the Ascidians, however, are too rare and uncertain in the fossil state for profitable examination in this paper.

Other organisms also show that boundaries between the three types of evolutionary series are not exclusive. The phylum Brachiopoda is in the main progressive, yet it includes many types in which the pedicle becomes obsolete as well as two of our most famous examples of stable series: *Lingula* and *Atrypa "reticularis."* Several mammalian series show degeneration in teeth and foot bones; most vertebrates show loss of bones in the skull; there is great degeneration of shell from primitive cephalopods to squids, the most abundant and efficient living members of their class. Such contrasts and combinations probably indicate that while degeneration *per*

se may be a genetically simple process, it commonly is combined with other processes which either give it no element of unfitness or even increase the fitness of degenerate types. If this is true, evolution is more complicated than either geneticists or paleontologists generally have supposed.

There remain those series, already mentioned, in which apparent progress is combined with giantism, elaboration of structures and ultimate or even imminent extinction. Variously interpreted as examples of racial senility, Buffonian adaptation, "law" in evolution (nomogenesis), slow modification of genes into successive multiple allelomorphs, and as results of natural selection acting to the advantage of the individual but defeat of the race, their significance is far from clear. I shall treat them as progressive series subject to the third and fourth of these factors, chiefly because such interpretations are virtually untested paleontologically and because they seem at least consistent with the data of cytogenetics.

Progressive Series

Plants

Two factors hamper interpretation of evolutionary series in fossil plants. One is the generally fragmentary condition of their remains, coupled with discontinuous representation in the geologic column. The other is that the anatomy of plants, even more than that of the higher animals, is unsuited to preservation of phyletic records in ontogeny.

There is no reason, however, to suppose that the genetic processes of plants in the geologic past differed greatly from those in the present. Specific differences are comparable throughout; if as Babcock has maintained (Babcock, 1931) gene mutations are inadequate to explain them to-day, we confidently may postulate chromosomal variations and polyploidy in the past. The comparative ease with which the latter may originate in

plants, both through vegetative propagation and hybridization, considerably simplifies the problem presented by substantial discontinuities and periodically rapid evolution. If many allopolyploids arising through hybridization had the qualities of the rice grass, *Spartina townsendii,* they account for many of the floral changes for which it has been customary to invoke environmental modifications. Another instance of hybridization producing results that among fossils would attract attention is Lotsy's cross between two snapdragons (*Antirrhinum*), in which the second generation flowers were referable to *Rhinanthus* (Lotsy, 1916: 128).

It is customary to attribute the accumulation of either mutations or transmutations to the benign influence of natural selection. It is conceivable, however, that slackening of selective pressure has been of greater importance in the origin of radically new types. Thus the rise of early land plants in the Silurian, of coal floras in the Devonian and early Carboniferous, and of modern floras in the Cretaceous, are readily understandable as outbursts of radical variation under lenient as well as new conditions, but less so if we picture them as subject to increased selection. The greater ease of transmutation in plants, already commented on, probably accounts for the fact that in each of these cases floral evolution preceded faunal. Yet delayed correlation exists: witness the elaboration of insects, amphibians and primitive reptiles in the Permo-Carboniferous, of swamp-feeding dinosaurs in the early Mesozoic and of mammals in the Paleocene-Eocene. In the last case, however, decline of reptiles rather than expansion of certain plants was the critical factor which relieved a potentially varied race from too stern selective repression.

Animals

Evolutionary series among both marine and continental animals show the effects of periodic diminution of

selection, thereby casting doubt on the familiar view that geologic revolution favors spread of new types. The revolutions closing each era, each period and many epochs doubtless eliminated archaic lineages already weakened (Tolmachoff, 1928), but there is little evidence that they directly favored either the development or establishment of new types. These remained incipient and rare until the return of stability when, on expanded lands or in widespread seas, they found a chance to develop.

Nor were the resulting lineages necessarily more fit, even to new habitats, than those which had disappeared. A comparison of shale-sea faunas of middle Ordovician, late Ordovician, middle Silurian, middle Devonian and late Devonian epochs[2] shows distinct assemblages of species, genera and larger groups, but similar types (life-forms in a loose sense) throughout. *Strophonella* of the latest fauna fills the niche of *Strophomena* in the earliest; massive corals in the Devonian replace massive bryozoans in the Ordovician; branching bryozoans, rugose corals, elongate cephalopods and crinoids occur throughout. One gains the impression that taxonomic groups are distinct because their ancestors have been distinct, not because of environmental demand for superior fitness. By the only criteria we have (abundance, length of time-range) *Sinospirifer* was less fit than *Platystrophia, Strophonella* than *Strophomena, Tabulophyllum* than *Streptelasma.*

There also is little evidence that each specific gene complex was so delicately balanced as to render the survival of mutants unlikely. Most of the specific differences in contemporary or nearly contemporary groups of *Spirifer, Atrypa, Platystrophia, Schizophoria* and many other brachiopods are comparable to characters produced by mutation. Yet their possessors lived, prospered and produced divergent descendants together. Here we have

[2] Decorah, Cincinnatian and Richmond, Waldron, Hamilton and Hackberry formations, respectively.

evidence of organic isolation and of successive modifications, perhaps in the form of multiple allelomorphs—but not of selection on any recognizable basis.

Still more conclusive are those lineages which pursue parallel but not contemporaneous evolutionary courses, all of which end in extinction. They may produce conspicuously "new" types, as in the Lituitidae and many lineages of ammonoids; they may present little more than elaborations, as in the Cheilostome bryozoans; they may be only degenerative, as in lineages of *Spirifer* to be reviewed. In any case, their progress ends in extinction, whose imminence is in roughly direct proportion to the degree of morphologic evolution. When evidence of environmental change is lacking (as it commonly is) and divergent or homoplastic lineages occupy identical localities and strata, we are forced to conclude that the environment tolerated evolution which resulted in progressive unfitness. Only when it became extreme, often betraying physiologic weakness, did extinction occur.

Such tolerance of variation, even when harmful, would result in great diversity and acceleration of evolution, even were changes strictly heterogenetic. But changes are not heterogenetic except in a very limited sense; commonly they have been the reverse. There are lineages of corals, cephalopods, brachiopods, trilobites, reptiles and mammals which can be made heterogenetic only if we suppose that all but one restricted type of heritable variation either was lethal, or resulted in death before recognizable hard parts were formed. The hypothesis is so improbable that it must be rejected—and limited (orthogenetic) variation is the only reasonable alternative. Thus by another route we conclude that the genetic factors outlined by Dr. Demerec have been at many times and in many lineages more effective than he supposes.

All this is not denial of natural selection; it is a suggestion that selection is neither so inevitable, so rigorous nor so consistent in even temporary improvement of the

race as it commonly is thought to be. In fact, it may take the opposite course and by piling up characters of advantage to the individual but harmful to the race produce extinction in even stable environments (Haldane, 1932, 119–124).

This hypothesis is most readily tested by gigantic horned, spined or armored races ranging from mollusks to mammals. Characterizing many evolutionary series at many times, reaching its acmae in lineages which soon became extinct, while smaller, less specialized relatives often survived, evolutionary giantism in bulk and armament must be considered the result of racially harmful selection—if selection is invoked at all.

The case is simplest in such lineages as the Ceratopsia (dinosaurs) and Brontotherioidea or titanotheres (mammals). In members of rare or scattered races, such as the early ceratopsians and titanotheres, individuals competed with members of other groups and with the inorganic environment. But when the relatively dense populations of the late Cretaceous and lower Oligocene were reached, members of each species and its close relatives also competed with each other. Individual success went to the rapidly growing, the strong, the effectively armed. The resulting selection doubtless helped in the accumulation of variations making for giantism, but it was racially beneficial only if the huge descendants were as capable of self-maintenance and reproduction as were their smaller contemporaries. That they were not is evidenced by extinction prior to, or without, substantial environmental change. The titanotheres decreased in both number and variety through the epoch of their evolutionary acmae; while imported disease may have completed their extinction, it hardly was responsible for such long-term decline (Scott, 1913: 312).

Intraspecific selection, in the form of a struggle for space, may have the same effect in plants, sponges, corals, bryozoans and other sessile animals of closely packed

associations. The most likely result would be giantism, with spines or heavy armor secondary or absent.

Intraspecific selection, however, does not account for such elaborately protected, yet inoffensive animals as *Spondylus,* the Antiarchi, stegosaurs, ankylosaurs and glyptodonts. Competition between members of a species would not make such armor valuable; if advantageous, it was so with reference to other, predatory associates. And again, its acmae are indices to imminent extinction, for which environmental changes do not often seem sufficient. Again it appears that if selection controlled the development of armor, it also determined unfitness.

Such evolution of incapacity is inconceivable on the hypothesis of invariably rigorous selection, permitting the establishment of new types only when the environment changes. But we have seen that many environmental complexes, especially during periods of widespread seas or of lowlands with equable climates, were tolerant rather than repressive. Granting ordinary efficiency to phyloephebic members of a lineage, there was opportunity for considerable decline in fitness before extinction ensued—especially if, as postulated, unfitness accompanied the development of characters individually advantageous.

It is not clear, however, that direct and indirect selection meet all phases of the problem. Large brachiopods (*Stringocephalus*) of the Devonian, giant ammonoids (*Parapuzosia*) and coiled pelecypods (*Gryphaea, Exogyra*) in the Cretaceous and early Eocene, and huge sauropods of the Jurassic seem to contradict any hypothesis of selection by their specializations in bulk or form. Still more puzzling are lineages of cephalopods characterized by coiling and uncoiling of the shell, elaboration and later simplification of ornament, etc. Even the titanotheres, here treated as possible examples of selection, are regarded by Haldane as organisms in which "the evolutionary process somehow acquired a mo-

mentum which took it past the point at which it would have ceased on a basis of utility." On a purely speculative basis he suggests (Haldane, 1932a: 108) that "we are dealing here not only with the accumulation of numbers of genes having a similar action, but with the very slow modification of single genes, each changing in turn into a series of multiple allelomorphs."

Haldane thinks that in these cases selection has favored certain modifications "at the expense of others"; but in a paper (Haldane, 1932) devoted to such series as the ammonoids, in which there is pronounced recapitulation, he suggests processes by which the time action of genes may be so delayed as to result in orthogenetic evolution. From this it is a short and (paleontologically) a reasonable step to modification of the genes themselves, by which selection would be reduced to a purely negative rôle. It seems clear that retardation, while significant in recapitulation and certain forward steps in evolution, is inadequate to explain the development of *Lituites,* of both involute and evolute ammonoids (Lang, 1919), and of certain lineages among corals (Carruthers, 1910).

To summarize: many progressive series among fossil animals may be interpreted as the results of selection acting upon genetic variations of known types, especially if we admit that selection may produce racial unfitness. In many series, however, variation appears to have followed definite trends or to have occurred within such narrow limits as properly to be termed orthogenetic. Even in series such as the Decapoda, Equidae and Elephantidae, such trends seem to have accompanied and dominated selection, by determining the variations on which it might operate. The fact that few if any animals whose genetics are known belong to series now progressive makes interpretation difficult; yet the very nature of these fossil series places greater emphasis on genetic factors of evolution than do current laboratory or mathematical studies.

STABLE SERIES

The paleontology of these, especially among animals, has been reviewed by Ruedemann under the title of arrested evolution (Ruedemann, 1918, 1922). He shows that from a phyletic view-point the organisms composing them may be divided into persistent radicles and persistent terminals. The first are truly primitive, ancestral stocks from which numerous shorter-lived branches repeatedly have been derived. The second are types that arose after the group was well advanced, perhaps even at or past its acme. They show specialization and in many cases appear to have survived by virtue of protective structures, functions or habitats, while the persistent radicles "are frequently dominant in the very seats of war."

Analyzing genera listed in a familiar taxonomic textbook (Zittel-Eastman, 1913), Ruedemann concludes that persistent groups are more common among lower classes and subclasses than higher, among marine animals than terrestrial, sessile types than vagile. They are specially abundant in groups that reproduce by division and buding, such as foraminifers, sponges, corals and bryozoans. Outstanding persistent (stable) groups among land plants are the horsetails, club mosses, cycads and ginkgos.

Scattered data on the chromosomes of persistent, morphologically stable groups are available. The gymnosperms, including cycads, ginkgos and conifers, have in the main 12 pairs of chromosomes (Hurst, 1932: 58). The short-horned grasshoppers (Acrididae) have changed little since the early Tertiary; of 800 species examined by McClung and his associates, the males as a rule have 23 chromosomes and the females 24. Poplars and willows, which range from the Jurassic and late Cretaceous with notable stability of leaf characters, have 19 to 114 chromosomes (Blackburn and Harrison, 1924). The Diptera, divergent but hardly progressive since the

Mesozoic, also show varying chromosome numbers (Metz, 1914–1923) even within genera. We must conclude that chromosome number is not a controlling factor in stability.

Turning to changes, most of which fall within the limits of speciation, we find that a large portion are on such small scale that they may be assigned to gene mutations. Thus brachiopods of the persistent Linnean "species" *Atrypa reticularis* (Linn.), within the comparatively thin upper Devonian formations of Iowa, show stable yet minor differentiation which in several lineages involved loss of characters (Fenton and Fenton, 1935). Comparable variations, too minute for taxonomic recognition, occur in many lineages of fossil invertebrates.

Other variations, however, are too great to be assigned to gene mutation. They probably arose through changes in chromosome constitution by loss or rearrangement of parts, or through changes in chromosome number—as in *Drosophila.* Large species of *Atrypa* with broadly lamellose shells seem to be exaggerations of small ones of older strata, a relationship that suggests polyploidy. In *Atrypa,* at least, only degenerative (genic?) variations seem to be orthogenetic.

Since polyploid development is most frequent in plants that fertilize themselves or reproduce apogamously, it is interesting to find that many large members of stable series occur in animals capable of reproduction by budding. Many species of sponges, corals and bryozoans of the geologic past may have arisen in this manner; but when preserved in direct combination of ancestor and descendant, they would appear as physiologic fluctuation. The importance of the latter in stromatoporoids, a group of marine monactinellid sponges, has been established (Twitchell, 1929): structural changes are so great that they might mask gaps of even generic rank. Paleozoic corals, which might give significant evidence, are too nearly unknown in both taxonomy and morphology to be

of aid. Yet in sections of apogamously reproducing specimens Mrs. Fenton and I have found differences which may be of genomic type, and polyploid origin for some gigantic forms with two or more times the ancestral number of septa seems probable.

I have avoided Ruedemann's term "arrested" because it suggests cessation of evolution, combined with survival. Even in such persistent radicles as *Lingula* this is not quite the case, since specific differentiation has continued through eras. It is even more marked in *Atrypa,* where conditions seem to have been very close to those presented by the living *Drosophila.* In fact, this comparison—as well as the very apparent stability of the Diptera as a whole—is the basis for assigning that genus to the group of stable series, with the conclusion that its genetic variability furnishes a reliable analogue of that in many fossils.

DEGENERATIVE SERIES

In long-term evolution of organic groups, degenerative series are less important than others. Not only have they failed to produce radically new organisms or (except in the case of parasites) conquer basically new environ-

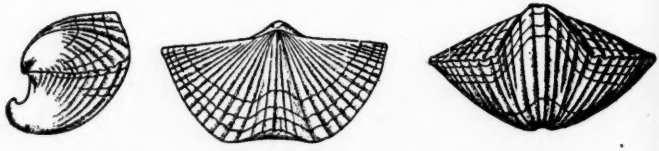

FIG. 1. Primitive shell shape in the *Spirifer varians* gens. Note the many radiating plications and acutely angular extremities. ×1.

ments; they characteristically have failed to survive through long periods or reach great numerical abundance.

Despite these limitations, degenerative series have great theoretical importance. They offer exceptional opportunities for determination of the nature of genetic

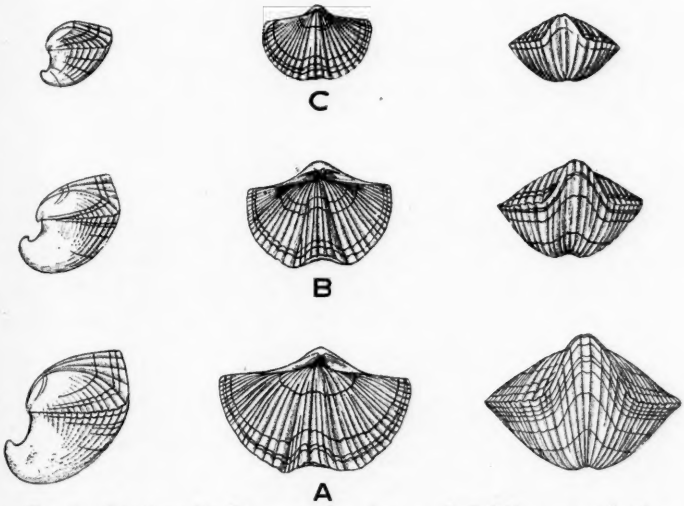

FIG. 2. Stratigraphically successive forms of *Spirifer approximatus*, showing change in gross characters while ornament remains stable. ×1.

changes, their physiologic backgrounds and their practical results. When traced in adequate collections, they furnish reliable evidence on the directions taken by genetic changes, deal concisely with the vexed problem of recapitulation and remove many possibilities of complication through selection. In short, if any fossils are susceptible to genetic interpretation, those belonging to these series offer most reliable data.

Instead of attempting to review a large number of degenerative series, I shall deal with three found among brachiopods of the advanced but not elaborately specialized genus *Spirifer*.[3] They chiefly occur in the lower member, 63 feet thick, of the late Devonian Hackberry formation, in strata which indicate rhythmic rather than progressive environmental changes, and they occupy a common area. Two are closely related, falling within the

[3] Unlike modern telotremate brachiopods, these seem not to have been permanently anchored by their pedicles. For a discussion of their orientation and habits, see Fenton and Fenton, 1935a.

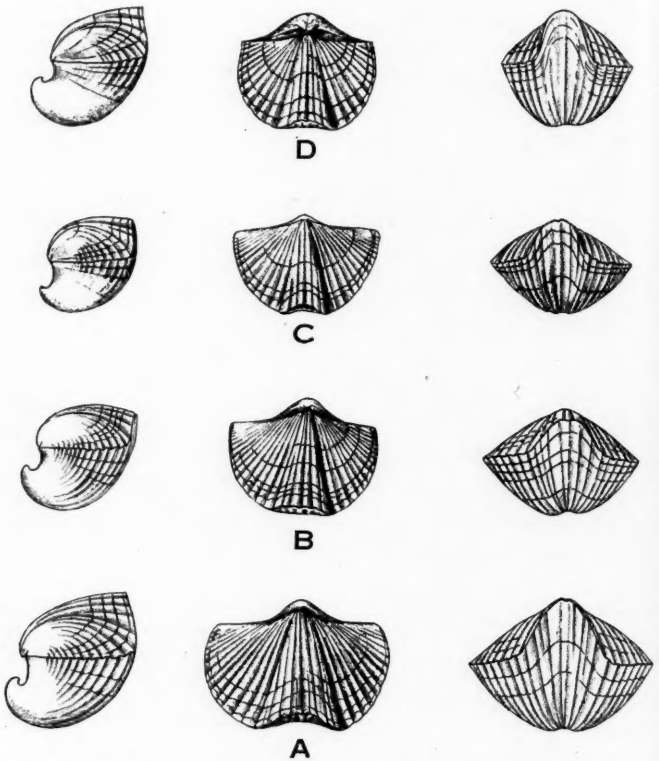

FIG. 3. Stratigraphically successive members of *Spirifer major*. Ornamentation of the earliest (A) is a modification of that seen in Fig. 4A; it changes little in later forms. ×1.

section *Aperturati* of Hall and Clarke; the third, still more closely knit, belongs to the relatively primitive subgenus *Choristites* Fischer.

Study of these groups began as taxonomic revision, but it soon became evident that taxonomic and lesser units, when arranged stratigraphically, automatically formed apparently determinate evolutionary series that were distinct, parallel or homeomorphic, degenerative and noncontemporaneous. A new collection of some 6,000 specimens gave identical results and emphasized another fac-

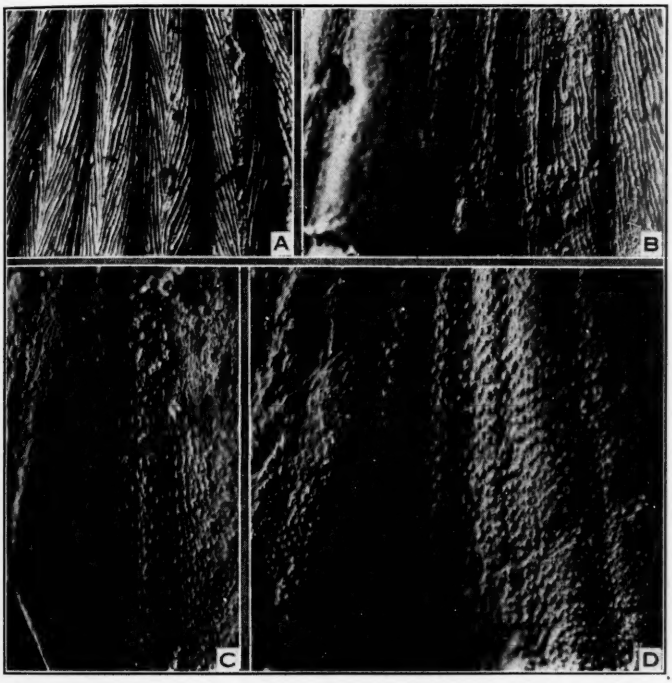

FIG. 4. Types of shell ornament in the *Spirifer varians* gens. A, primitive striae; B, constricted, nodose striae; C, club-shaped markings from constricted striae; D, scattered pustules. B–D occur in independent lineages and are not contemporaneous. × 8.

tor: close correlation between degree of degeneration and imminence of extinction.

In the *Spirifer varians* gens[4] the primitive shell is wide, with angular extremities, deep mesial sinus, high fold and free opening for the pedicle. The surface bears minute, continuous, oblique ridges which for convenience have been called striae. Evolution involved narrowing,

[4] A gens is a monophyletic group of taxonomic species, possessing numerous characters in common. It roughly corresponds to the "collective species" of Waagen, but the stability of its members suggests that it exceeds the limits of "genetic species." These may find their equivalents in subgentes.

and real or proportionate lengthening of the shell, blunting of extremities, weakening and diminution in number of plications and recurving of the beak until in some cases it hampered or prevented operation of the pedicle. Striae coarsen and in many cases become subradial or flexuous; they also disintegrate into nodose ridges, then

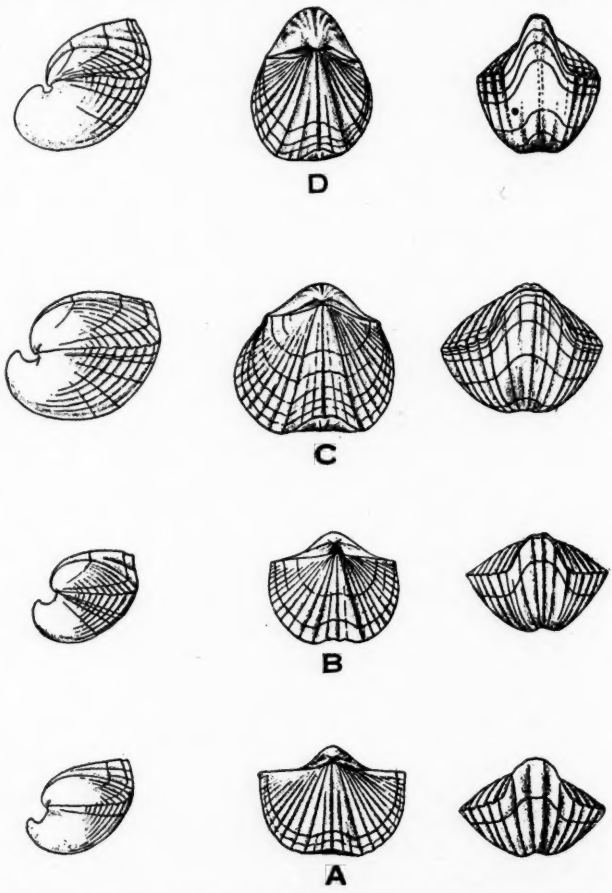

FIG. 5. Degeneration of form and plications in the *Spirifer gravis* section, *S. varians* gens. It is accompanied by breaking of striae, first into club-shaped markings and finally (D) into pustules. ×1.

rows of club-shaped pustules and finally (if the lineage survives) into round pustules which lose all traces of striate pattern.

Of the two character-groups, those involving surface

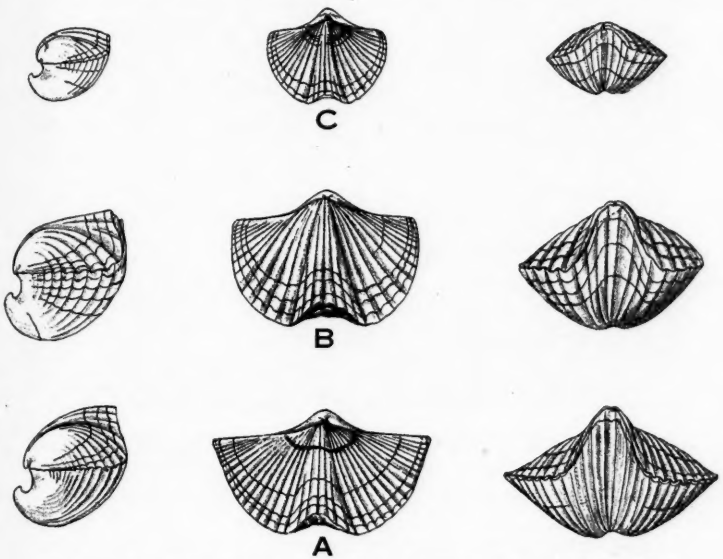

FIG. 6. Successive forms of *Spirifer granilineatus* in which initial development of highly nodose striae (A) is accompanied by slight lengthening, but no degeneration of plications. Later changes are shown in B and C. ×1.

ornament seem the more nearly fundamental. Figs. 2–3 show stratigraphically successive members of two limited species, in each of which surface characters remain stable, while those of shape and plications show degeneration. Degeneration of ornament *within* a lineage, however, is accompanied by gross changes—a fact well shown by Fig. 5, in which the initial member (A) has well-marked striae comparable to those of Fig. 4D, while the terminal one (D) bears pustules.

Radical departures, however, violate this correlation. Thus the species (*S. granilineatus*) of Fig. 6A is characterized by extreme nodosity of striae, yet it retains al-

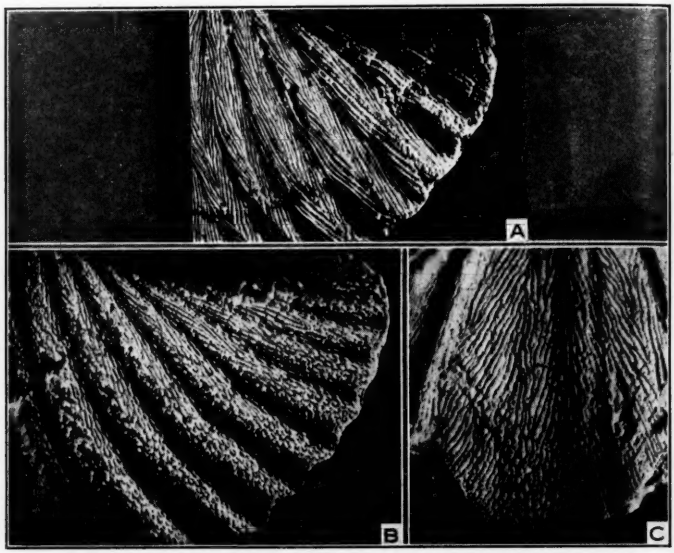

FIG. 7. Surface ornamentation in the *Spirifer obliquistriatus* gens. A, an early and primitive member; B, an intermediate; C, an advanced (late) member. ×8.

most primitive shape, with primitive strength and abundance of plications. But in the successive forms B and C, whose ornament shows only slight loss of striate pattern and rounding of pustules, there is pronounced degeneration of gross characters.

Similar trends appear in the *Spirifer obliquistriatus* gens, which first appears in strata almost 24 feet higher than those containing the oldest specimens of *S. varians*. Primitive (early) ornamentation consists of oblique striae, shown in Fig. 7 A; advanced degeneration, in two lineages, results in elongate to rounded pustules, Fig. 7 B–C. Accompanying, or following these are trends involving reduction in width, number and ultimately strength of plications as well as shell-size. These reach their extreme in shells from Arizona which, on geologic probability, lived considerably later than those of Iowa.

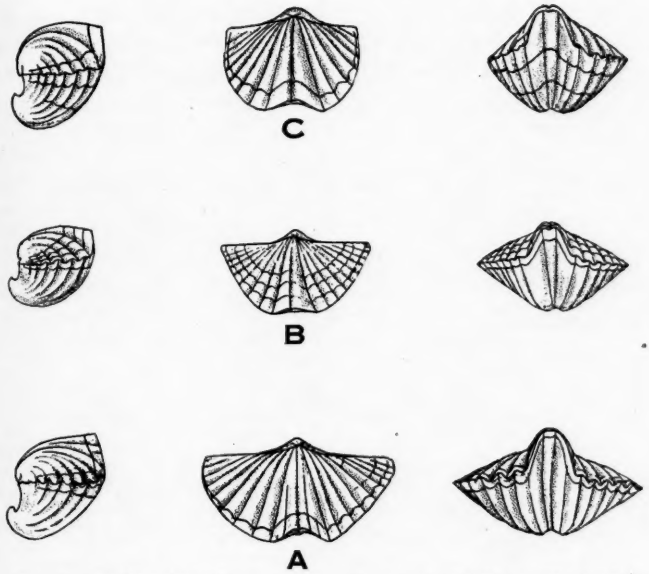

FIG. 8. Stratigraphically successive forms of *Spirifer obliquistriatus*. Almost identical sequences are found within 5 of the 11 species and subspecies in this gens, as well as in 6 minor lineages which they in turn compose.

Degeneration of striae, however, is most advanced in Iowa material.

These shells show another feature characteristic of both gentes: recapitulation of adult characters in shape and ornament that is detailed and precise, with slight disturbance even by acceleration or tachygenesis. Even in forms too detailed for taxonomic recognition, such recapitulation serves both to retrace phylogeny and—in a very practical way—to anticipate it. Thus, when the first specimens of *Spirifer retiferus* were found, none were known in which adult characters were the elongate pustules and broken striae which appear in successively earlier shell areas in that advanced form (Fig. 7 C). Search was made in appropriate strata and an intermediate, *Spirifer inceptoides* (Fig. 7 B), was discovered,

linking the species *obliquistriatus* and *retiferus*. With this hint, later strata also were examined and a terminal, more definitely pustulose subspecies, *S. retiferus terminus,* was found. It, in turn, shows form changes

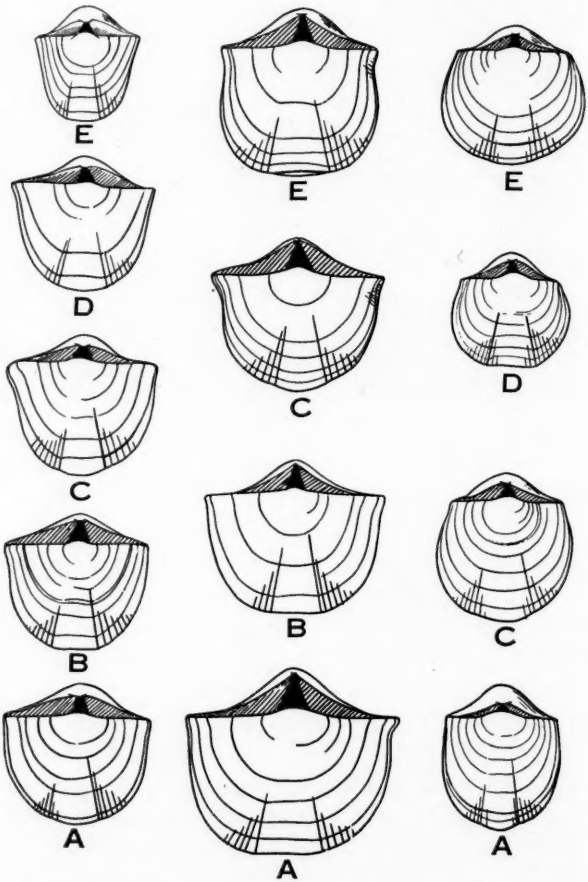

FIG 9. FIG. 10. FIG. 11

FIGS. 9–11. Evolutionary trends in *Spiriferi* of the *S. hungerfordi* gens, form A being primitive in each group, though well advanced in the larger series of which these groups are members. Ontogenies indicated by outlines of growth lamellae. ×1.

comparable to those of Fig. 8. The process was repeated in several other lineages and resulted in the discovery of both intermediate and primary members.[5]

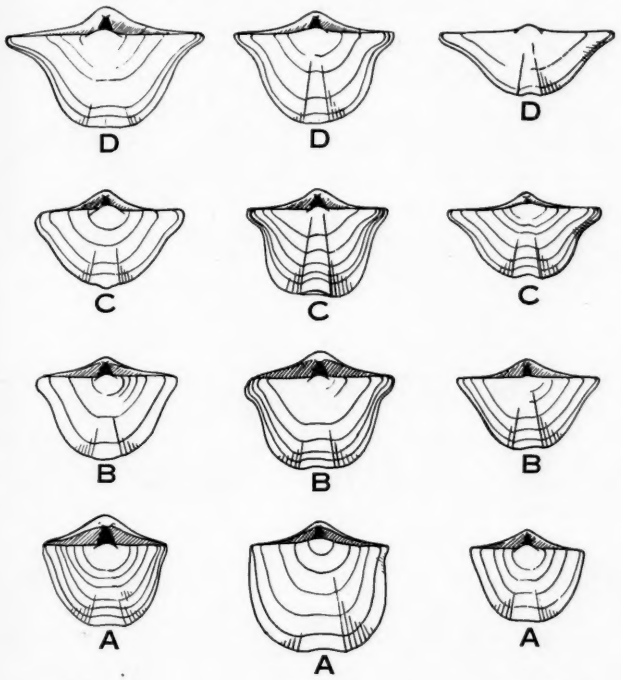

FIG. 12. FIG. 13. FIG. 14.

FIGS. 12–14. Evolutionary trends in three wide members of the *Spirifer hungerfordi* gens. Terminal forms (D) do not repeat adult outlines of their immediate predecessors. ×1.

Evolutionary trends in the *Spirifer hungerfordi* gens are shown by gross characters only, minute ornament be-

[5] This utilization of recapitulation to "predict" undiscovered fossils and their approximate horizons is commonplace in paleontology and constitutes a strong, but rarely mentioned, argument in favor of the principle. I have elsewhere offered detailed evidence of its validity in these *Spiriferi* (Fenton, 1931a), while other authors (*e. g.*, Cumings, 1909, George, 1933) have dealt with larger arrays of evidence, and with misunderstandings which seem to underlie most adverse neontologic criticism of recapitulation.

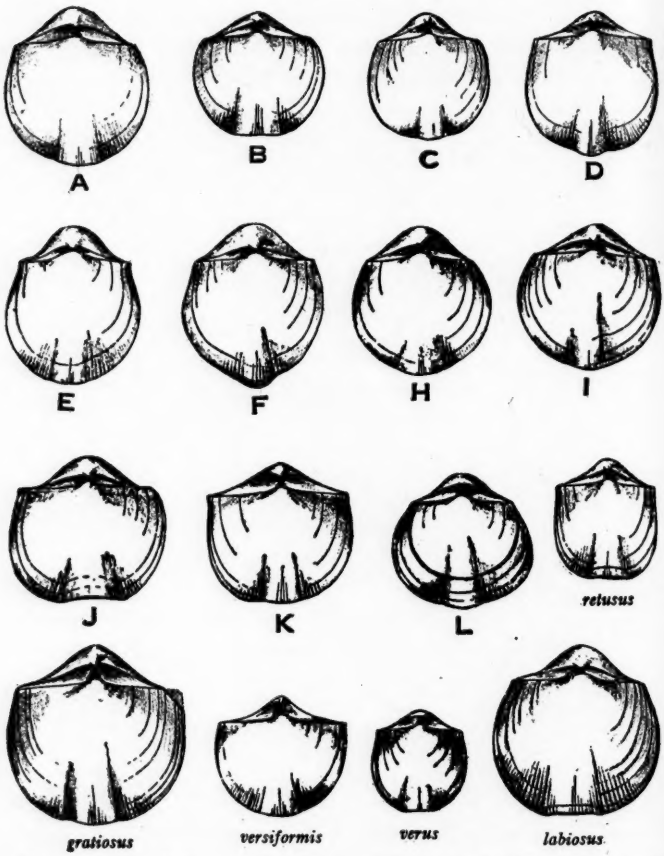

FIG. 15. Essentially contemporary members of *Spirifer hungerfordi*, which apparently bred true. "Forms" of *S. hungerfordi* indicated by letters; subspecies by names. Since the latter themselves are divisible into forms, only the earliest (A in each case) is shown. ×1.

ing stable. There is less uniformity of type than in the preceding gentes: some lineages show evolution, which primarily involves lengthening of the shell without proportionate lateral growth (Figs. 9–10); others show lateral enlargement to be dominant (Figs. 12–14); a few show the former (culminating in shells like that of Fig.

11 A) followed by failure of elongate growth (Fig. 11 D–E). Especially in *S. hungerfordi* itself there are numerous nearly contemporary lineages which seem to have arisen, not orthogenetically, but directly from the ancestral type. Minute similarities, extending through ontogeny, indicate that they are mutants which bred true rather than repetitions of similar mutations. Occupying a limited habitat, through a single time-division, they seem to form another example of differentiation under environmental toleration or relief from selection. Some of these soon became extinct, but others established line-

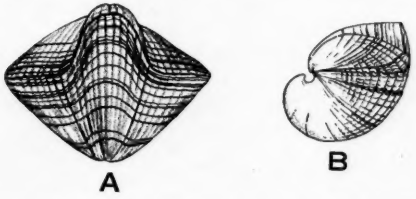

FIG. 16. Growth lamellae in late members of the *Spirifer whitneyi* and *S. varians* gentes. For lamellae of earlier members of these lineages see Figs. 17 D and 5 A–B. ×1.

ages divisible either into "forms" too restricted for naming or into subspecies.

Recapitulation within each minor lineage may be detailed (Figs. 9–10), or it may involve crowding and loss of stages. In some cases, the early ancestral characters disappear first—the phenomenon called tachygenesis by Hyatt. In others (Figs. 11–14) early characters remain, but intermediate ones are lost. Thus shells of Fig. 11 E repeat adult outlines ancestral to A, but not those of A–D, and there is similar omission by forms D of Figs. 12–14. In each case, characters omitted are those which are inconsistent with adult shape in the terminal member, a situation paralleled in various lineages of cephalopods (Trueman, 1922).

These phases of evolution are morphologic; others, in these three gentes and the associated *Spirifer* (*Sino-*

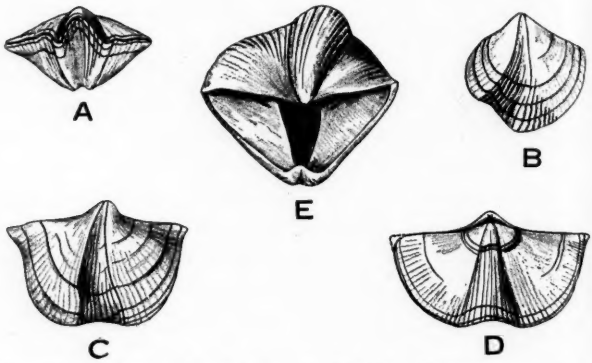

FIG. 17. Injury and repair in the *Spirifer whitneyi* gens. D is the earliest member of the group; E the latest. ×1.

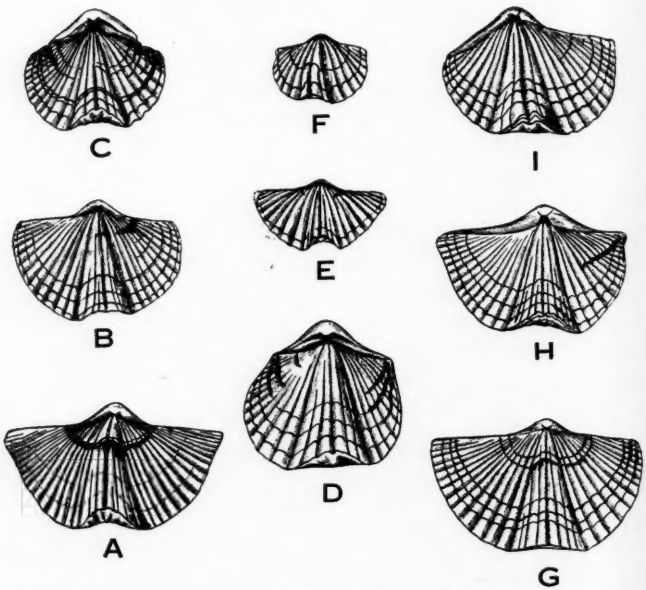

FIG. 18. Injury and repair in the *Spirifer varians* gens. A, E and G are early and primitive within their lineages; B–D, F, H–I are phyletically advanced and occupy higher strata. ×1.

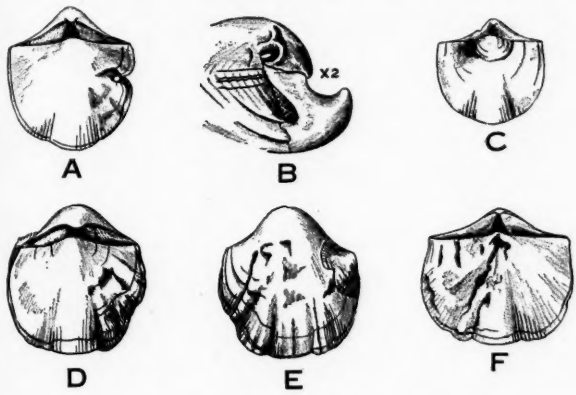

FIG. 19. Injury and repair in the *Spirifer hungerfordi* gens. A–C early members, D–F late ones. In A–B, both valves surround a bryozoan column, with little disturbance of adult symmetry. In C, one extremity of the shell was crushed, its fragmented edges being incorporated in new growth. ×1.

spirifer) *whitneyi* gens are physiologic. In each of these groups, early members show considerable ability to repair shell injury and establish normal outlines and symmetry. They also bear few growth lamellae, which rarely are thick. Late members of the same lineages, however, show abundant lamellae, indicating frequent disturbance or cessation of shell growth. Injuries to

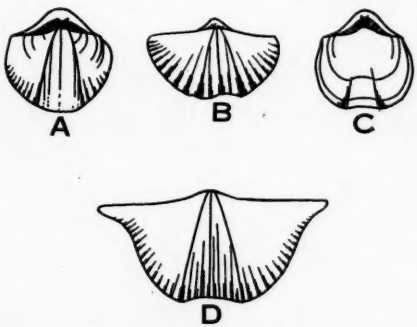

FIG. 20. Contemporary members of four gentes in the genus *Spirifer*. A is phyloephic, B phyloneanic, C incipiently phylogerontic, D phylogerontic, near extinction. ×1.

shell and mantle, even if less conspicuous than those repaired by early members, result in gross distortion of the adult shell. It seems clear that progressive differentiation and phyletic age were accompanied by reduction in ability to endure environmental disturbance or to repair damage.

In these degenerative series, as in the armored, giant and elaborately ornamented lineages, evolutionary specialization at first may be accompanied by increase in numbers; but this soon gives way to decrease terminating in extinction. And as in those series, the imminence of extinction is proportional to the degree of evolutionary differentiation (degeneration).

It seems impossible to explain these series on a selective basis. Were variations advantageous, or correlated with others of advantage to the race, extinction so orderly, so inevitable in environments changing rhythmically (not progressively) would be impossible. Since most lineages show reduction in size, competition for space is eliminated; there is no basis on which to postulate selection of characters individually advantageous but racially destructive. There is not even the faint hint of Buffonian variation which some authors (Hyatt, 1894; Dunbar, 1924) have built into elaborate hypotheses of evolution in other phyla. We are forced to conclude that evolution in these brachiopods was controlled internally, even when it involved unfitness; that it followed definite courses or trends, and that environment merely eliminated lineages already in advanced decline.

Identical character-sequences in ontogeny and phylogeny suggest a hypothesis of racial life cycles or racial senescence. Though familiar for decades, this concept generally has been combined with acceptance of Buffonism, or has relied on such doubtful parallels as "racial second childhood." Here we find no such complications; to insure their elimination, this theory was phrased:

"Stages in the life history of a race may approximate

those of its individuals, and in such cases, rest upon the same physiologic basis. Changes involved in racial origin, in such series, find their cause in genetic variations which increase the metabolic rate; those of differentiation and decline (racial senescence), in heritable variations which reduce that rate." (Fenton, 1931)

"Decrease in metabolic rate may operate directly or through the medium of specialized organs. . . . It gives rise to morphologic and physiologic changes which are limited in type, which show distinct (through not necessarily direct) evolutionary trends, and which are cumulative. The metabolic decrease itself pursues a trend which, though it may be delayed or diminished, apparently is not halted, so that its ultimate termination is senility and extinction." (Fenton, 1931a: 104)

Though derived from a suggestion by Child and based on his conception of senescence (Child, 1915), this theory is not limited thereto. Its essential point is that evolution may, and commonly does involve changes which in both physiologic and morphologic results are identical with senescence, whatever the nature of that process may be.

The question now arises: can such a theory of phyletic senescence be made intelligible in terms of genetics?

A concrete example is the best test. Let us select the lineage beginning with *Spirifer obliquistriatus* and reaching its clearest terminal in *S. retiferus* (Fig. 7 C). It may be supposed that shell ornament, like color in many living animals, was determined by several genes—for convenience represented as 3. Because every change, both ontogenetic and phyletic, is degenerative, it appears that the functionality of these genes diminished through successive growth stages and generations. No new characters were introduced.

Fig. 21 is a diagrammatic representation of the situation. Consider it first as the ontogeny of *Spirifer retiferus:* during early life (Stage A) all genes are

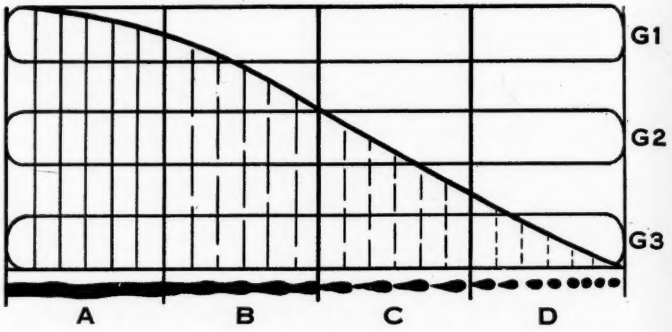

FIG. 21. Diagram representing ontogeny and phylogeny of *Spirifer terminus*. G1–G3, genes responsible for surface ornamentation. A–D, successive organismic environments (lined areas) and morphologic characters (black).

functional, developing coarse striae. In succeeding stages (B–D) the organismic environment is such that gene functionality diminishes, until in D only one is operative and that with diminishing effectiveness. Hence there is progressive degeneration in surface characters, from striae through constricted striae (B) and elongate markings (C) to pustules (D).

"Organismic environment" is but another term for the complex of genetically determined conditions in which selected genes produce selected results. To extend this hypothesis from the individual to the lineage we must suppose that genes 1–3 are present throughout; that other genes in phyletic stage A (*Spirifer obliquistriatus*) enable them to function throughout life; that in successive stages these other genes are so modified as to produce limitations comparable to stages B–D in the individual. In short, genes which determine physiologic rather than morphologic characters undergo changes which substantially are senescence.

What might cause such change? Not the external environment, whose effects would be rhythmic and essentially contemporaneous in all lineages; not selection,

which saves or destroys the results of gene functioning but leaves the genes themselves untouched. The one likely factor is the organism itself, which goes through a continuous and predetermined series of changes—senescence. It is true that each new generation involves a return to the beginning of the cycle; but as Child points out (Child, 1915: 463–464) we have no reason to believe that each new generation returns to exactly the same physiologic level attained by its most remote yet direct ancestor. Unless it does so, there will be gradual senescence of protoplasm during evolution, which the genes hardly can escape. Paleontology, with its countless extinct lineages and many instances of recapitulation, gives strong weight to Child's conclusion that "the facts point very definitely" toward such phyletic senescence.

Processes rarely were so simple as they seem to have been in the development of *Spirifer retiferus.* That species must have repeated, with exceptional precision, the organismic environments of its ancestors. Its descendant, the subspecies *terminus,* may have been less precise: at least, while striae, pustules and intermediate markings are present, they are distinguishably different from those of *retiferus* and its ancestors. Or there may have been one or more mutations, changing slightly the characters developed.

Such mutations seems to underlie most of the species and subspecies in these complex lineages. Evidence for control of characters by several genes rather than one pair is found in the similarities which persist through change, especially in early stages. Since there is no reason to suppose that mutation would affect only genes coming into operation during late neanic or ephebic stages, the numerous cases of recapitulation seem to indicate the physiologic control already postulated. Such control, with approximation of ancestral states growing shorter and less close with successive generations and

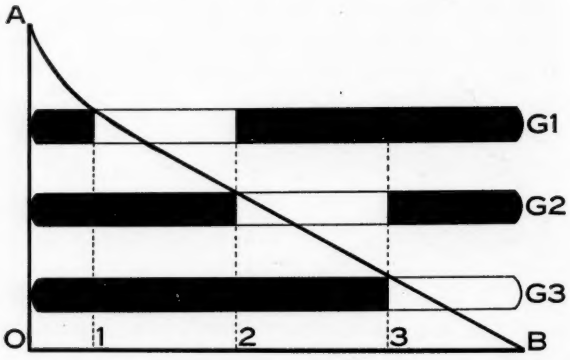

FIG. 22. Senescence controlling lipopalingenesis. G1–G3, genes functional in succession during ontogeny; A–B, changing physiologic states of senescence. Mutation of Genes 2–3 would give an ontogenetic series of characters 1, 2^m, 3^m, instead of the phyletic series 1, 2, 3, 2^m, 3^m—the letter m indicating the mutated genes.

ultimately disappearing, offers a hypothetical, but reasonable basis for tachygenesis.

Skipping of stages (lipopalingenesis) is a different problem. Here there seems to be either loss of genes, or progressive mutation in specific gene loci, while others remain stable. The latter hypothesis, which best seems to fill the requirements of the lineages in Figs. 12–14, demands greater autonomy of the genes than the stated hypothesis permits. Here, since characters seem distinct, we may suppose that each is conditioned by a specific gene-pair, which functions when the organismic environment permits. If Gene 1 remained stable while Genes 2 or 3 mutated, there would be elimination of the stages which they determined in past phylogeny, yet adequate basis for the repetition of characters determined by Gene 1.

Mutations of magnitude in genes which are effective at other than the final stage will produce, not skipping of stages, but cenogenesis. Thus a phyletic sequence might run 1, 2, 3, 3^m, 1^m; but the ontogeny 1^m, 2, 3^m would

give slight clue to such an ancestry. That cenogenetic variations of greater magnitude than this have occurred repeatedly is obvious; but they seem to have resulted in progressive rather than degenerative series.

In short, while it remains possible that such series as cephalopods, reptiles and mammals show racial senescence (as many authors have suggested), they include complications which at present lie beyond the scope of the theory here advanced. The combined factors of trends, degeneration and recapitulation, physiologic decline and extinction are its mainstays; until they or equally reliable evidence can be adduced for other groups, it seems unwise to extend the scope of the theory.

Conclusion

This paper admittedly is speculative, for reasons partly inherent in fossils, partly in the still imperfect state of genetic knowledge. Yet perhaps its most ambitious venture, into the genetics of phyletic senescence, is not more far-fetched than mutations, crossing-over and polyploidy appeared to those who met the early developments of genetics with the conviction that genes were fictitious and chromosomes artifacts. Though there is small chance that these paleontologic speculations will receive the convincing support accorded the theory of genes, they may suggest genetic avenues of approach to their central problems. Now that the conflict over discontinuity has ended, other questions wait; even tentative steps toward their answers may be of substantial value, in both extending the scope of genetic interpretations and suggesting lines of helpful paleontologic research.

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EVOLUTION OF THE CHROMOSOME CONCEPT[1]

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THE evolution of the chromosome concept has been both brief and direct. It started, of course, with Roux's brilliant suggestion of the significance of the chromosomes as indicated by their behavior in mitosis. Almost directly Weismann perceived in this a basis upon which to found his theories of the germ-plasm. It is interesting to note that early investigators entertained no doubts concerning the reality of chromosomes, while in later years, with much greater knowledge, it became the fashion among certain groups to deny their existence or to call them crystals or other fanciful names. In recent years, however, so great a body of concordant evidence from cytological and genetical sources has grown up that it is now about as reasonable to deny the existence of organisms as it is to doubt the presence and causal influence of chromosomes in life processes. Indeed it is most remarkable, in so short a period as the last half century, to have so firmly established such a biological generalization as the chromosome theory of heredity.

Weismann, himself, it may be recalled, was principally concerned with the problem of establishing a complete theory of heredity, and his attention was therefore fixed upon the ultimate units, or biophors, each of which would determine the character of some cell part, and of their aggregates into determiners of cell characters. These, each organized into ids, would contain all the determinants necessary to control all the characters of the species in development. Ids were not conceived to be entire chromosomes, and were never definitely identified with any part of the chromatin, but might conceivably correspond to the chromomeres. Each chromosome

[1] Address at the meeting of the American Society of Naturalists, Pittsburgh, December 29, 1934.

would be, therefore, a mass of similar units and all the chromosomes would be alike. According to this view, there could be no primary significance in the chromosome as a body. Parts of chromosomes or even whole chromosomes could be lost without thereby removing any essential controls for cell development. The relationship between a determiner and a cell character was assumed to be direct and simple. At a given time and place the determinant would produce its unit effect and thereafter would continue to act that rôle. The process was viewed as entirely performational in character. The germ cells, of course, were supposed to retain all their determinants and to constitute a continuous series, but the body's cells would become progressively simpler in their determinants until finally each would contain but a single kind.

While we are still at one with Weismann in the belief that there is special substance having a primary influence in heredity, continuous from generation to generation, identifiable with the chromatin, and made up of still smaller elements, we have quite other views regarding the manner of their integration and action. The first of these differences appeared when it was recognized that all the chromosomes are not alike. So soon as it was known that the group of characters called sexual are primarily determined by one particular chromosome, then Weismann's views of the common character of the ids and the uniformity of the chromosomes failed. Almost at the same time Sutton drew the parallel between chromosome phenomena in the germ-cells and the behavior of characters in alternative inheritance, including linkage, which assumption has been brilliantly demonstrated as true by the work of Morgan and his collaborators. The chance segregation and recombination of chromosomes in maturation and fertilization was proved by Carothers, and the constant linear organization of the chromosomes was established by Wenrich. All these facts made it necessary to reconstruct entirely the earlier views of chromosome

integration. Weismann himself, as we noted, does not appear to have given much thought to chromosome organization. He assumed, of course, an architecture of the germ-plasm, but the nature of this he did not consider at length. Investigators at the present time are most actively engaged in the demonstration of the internal structure of the chromosomes by combined cytological and genetical analysis. This constitutes one of the most brilliant exploits in biological history and confirms well the view of early cytologists like Pfitzner, Roux, Flemming, Strasburger and others who saw significance in the linear organization of the chromosomes and their precise division in mitosis.

While Weismann's views of chromosome structure were very different from the modern ones, it is perhaps in relation to the manner of their action that he departs most from present accepted theories. He could see no epigenetic element in the relation between determiners and characters. For each kind of cell he visioned a specific determiner, for each part of a cell its own biophor. By successive divisions these gradually sorted out until finally but one kind remained to control the activities of the cell. This predetermination scheme he recognized as in some way dependent upon an unknown architecture of the germ-plasm. In certain respects this is no explanation at all, for it merely says that each character is dependent upon a control and that these controls come successively into action as a result of an organization about which we know nothing. Concerning the significance of the integration of these controls into chromosomes, Weismann has little to say, except that they are combinations of ids, although not permanent ones. Of course this is just the opposite of what we now know to be true. The chromosomes are traced through the entire life history of an organism without any indication that they are progressively losing their component elements. Moreover, it is demonstrated that the manner of association of the determiners, or genes, into the chromatin elements

vitally affects their action. The chromosome is therefore a specifically organized body whose action depends upon three factors, *viz.*, the number and character of the elements contained in it, the relation of these to each other, and the relation of the whole to the rest of the cell and to the environment in which it is placed. The problem of explaining the performance of a hereditary mechanism is not to imagine the simple one of having a series of elements falling into action according to some predetermined scheme, producing each its peculiar effect at one particular time and place, but rather to conceive how this action can take place in the presence of an apparently unvarying series of units perpetuated entire in each mitosis. As guides to the understanding of this, we have the demonstrated facts that each character depends for its development upon the action of many units and that each unit has many effects. A chromosome influences many characters by reason of the elements it contains and the peculiar relations which they bear to each other. It is a fair inference that what is true of the entire chromosome holds also for its parts. It must also be supposed that these elements are continuously active in all stages of development and do not wait for a particular time or place to come into action.

In seeking to understand how the elements of a chromosome are related or genes are organized into larger nuclear aggregates, we encounter at a very early stage the profound problem of organization and differentiation. We can define the situation very concretely by asking the question, "What is the reason for the existence of such bodies as chromosomes—why could not the ultimate determiners act as independent units?" Since chromosomes are universally present in cells, it is certain that some important result is secured by their type of integration. Linkage is, of course, a very concrete and obvious resulting phenomenon, but there must be something of a more fundamental nature involved, otherwise there would not be the constancy of individual chromo-

somes through large groups and infinite numbers of organisms. There are many hints toward a solution of the enigma, but no compelling lead. It is perhaps not without significance that polyploidy is common in plants and more rare in animals; there may be meaning in the fact that some groups have many small chromosomes while others have a few large ones; it should be suggestive that numerical variation is common in certain cases and rare or absent in others; unusual chromosome associations, found throughout some groups, have a meaning if we could but grasp it.

But despite all the knowledge we have gained of chromosome characteristics and behavior we are still entirely in the dark regarding the reason for their existence as elements of cellular organization. This failure to comprehend the ultimate nature of chromosomes does not, however, hinder us from practical efforts to discover how they are organized and how they produce their results. There are two methods of attack upon this problem. One traces the relations between successive members of an organic series and associates changes in the behavior of the chromosomes with variations in body characters. This method is in the ascendancy at the present time and is producing most valuable results. There can be no question of the significance of the facts ascertained in this manner, although their limitations must be recognized. The embryologist points out that they do not contribute to our understanding of the processes of development and the paleontologist says they have little value in tracing phylogenies. While there may be some justification for the opinions quoted, it is equally true that any facts bearing upon the functions of chromosomes at any period in the life history of an individual or in a succession of individuals must eventually contribute to an understanding of the full course of their activities. Just because a developing concept is not completely applicable, at the time, does not lessen its importance in a final comprehension.

The second method of attack lacks the rapidity and spectacular character of genetical experimentation and has, at present, few followers. It involves the comprehensive analysis of the characters of a group of organisms together with a correspondingly extensive study of chromosome conditions in their germ-cells. There are many difficulties in so general an investigation and many years must pass before sufficient results can be obtained to warrant final conclusions, but their phylogenetic value will be great. Fortunately in the pursuit of ultimate aims many important and often unexpected by-products of fact and theory emerge to the great encouragement of the patient investigator. Certainly such thorough and searching studies are necessary before we can hope for an understanding of what has gone into chromosomes to make their peculiar activities possible. In the long range view the element of time must enter largely. After the manner peculiar to living matter, the experiences through which it passes produce changes which become incorporated into it. In a sense, time becomes a part of it—a material embodiment. From this point of view the chromosomes are time reservoirs, for in them are recorded the results of these temporal experiences, if they are to be permanent characteristics of the group. How definite and uniform this reaction is appears evident from the fact that throughout the species, genus, subfamily or family the same complex of homologous chromatin units governs the development of its characters. We are yet, however, in search of answers to the questions concerning the means by which these controls operate to condition the characters of the group, and the modifications they undergo in order to produce the many observed variations of the organic type. While we are thus unfortunately ignorant of the manner in which chromosomes act to produce their effects we have gone a long way from the simple "one to one" relation idea that dominated the theories of Darwin, Spencer and Weismann.

The view that determiners escape from the nucleus

into the cytosome and there produce each its specific effect seems to us a very crude conception of the intricate and balanced system of cell organization. On the contrary, we must think of the chromosomes as dynamic entities made up of specific elements, definitely organized in relation to each other and forming an integral part of a coordinated system, including the parts of the cell and its environment. It is assumed that these elements are in constant action during the life of the cell and that the exact nature of the act in each case depends upon circumstances, extrinsic and intrinsic, and so differs at every period in the life history of the organism. This is the present concept of the chromosome, and it departs widely from that indefinite and formal view of it which characterized the beginning of the fifty-year period whose close we observe to-night. We can only hope that the next fifty years may produce proportionately greater knowledge.

SHORTER ARTICLES AND DISCUSSION

LINKAGE OF THE GENES FOR NON-YELLOW (y) AND PINK-EYE (p_2) IN THE HOUSE MOUSE (*MUS MUSCULUS*)[1]

In a note in *Science* Roberts (1931) gave a short description of a new mutation in the house mouse, in which the eyes are pink, and black, brown and the yellow of agouti are diluted. The young from a mating of this new pink-eye mutant and the common pink-eyed mouse have dark eyes and intense color. The new pink-eye was tentatively called p_2 which for lack of a better symbol we are still using. The results of matings involving the new pink-eye gene (p_2) and the normal allelomorph (P_2) are given in Table 1. These results show that the difference between this new character and normal dark-eye is due to a single recessive gene.

TABLE 1

SEGREGATION OF DARK-EYE (P_2) AND PINK-EYE (p_2)

Mating		Total	Classes produced	
			Dark eye (P_2)	Pink eye (p_2)
$P_2p_2 \times P_2p_2$	Obtained	612	471	141
	Expected		459	153
$P_2p_2 \times p_2p_2$	Obtained	690	374	316
	Expected		345	345

A study of litters in which mortality occurred after birth and before the young were classified showed that there was no differential mortality in respect to the genotypic classes produced, so all the tables contain the data from both full and depleted litters. This new gene also dilutes the yellow of yellow mice to a light lemon color. When the common pink-eye gene (p_1) and p_2 are together in the homozygous state they have a cumulative diluting effect producing very light-colored coats much lighter than either one separately. In this respect they behave much in the same way as do blue dilution (d) and red eye (r) in the rat. When

[1] Contribution No. 47 from Division of Animal Genetics, Department of Animal Husbandry.

these two characters are present in the individual, color is much more diluted than it is when either one is present alone.

The following linkages according to the compilation by Keeler (1931) have been found in the mouse: (a) Albino (c), shaker (s^h), pink eye (p_1); (b) Rodless (r), silver (s^l); (c) Spotting (s), hairless (h^r); (d) Short ears (s^e), dilution (d).

He also suggests the possibility that agouti and white-belly instead of being allelomorphs are closely linked.

In the course of our studies evidence has recently been obtained showing a linkage between non-yellow (y) and p_2. Yellow was used instead of agouti because with yellow the phenotypic classes are more easily distinguished than they are with agouti. Heterozygous individuals of the composition $\frac{YP_2}{yp_2}$ were mated to homozygous recessives $\frac{yp_2}{yp_2}$. If these genes are independent, four phenotypic classes of offspring, YP_2, Yp_2, yP_2, yp_2 should be produced in equal numbers. The young were examined at least twice, fourteen to twenty-one days apart, in order to eliminate possible errors in classification.

Table 2 contains the results of the matings to test the linkage

TABLE 2

LINKAGE TESTS OF PINK-EYE (p_2) AND NON-YELLOW (y)

	Phenotypic classes				Parental combinations	New combinations
	YP_2	Yp_2	yP_2	yp_2	YP_2+yp_2	Yp_2+yP_2
Obtained ...	188	41	52	174	362	93
If independent	113.75	113.75	113.75	113.75	227.5	227.5

relation of p_2 and non-yellow. On the basis of these results the crossovers are 20.4 per cent. of the total. Of the 455 progeny from the backcross, 231 were produced from matings of YyP_2p_2 females and yyp_2p_2 males; 182 of these were non-crossovers and 49 crossovers, or 21.2 per cent. crossovers. From the matings of yyp_2p_2 females and YyP_2p_2 males, 224 were produced, consisting of 180 non-crossovers and 44 crossovers, or 19.6 per cent. (see Table 3). This difference in percentage of crossovers in the reciprocal crosses is not large, yet it is in line with other

TABLE 3
LINKAGE TESTS OF p_2 WITH y

Mating	Classes produced				Crossover classes	Crossovers per cent.
	YP_2	Yp_2	yP_2	yp_2	$Yp_2 + yP_2$	
♀ $\frac{YP_2}{yp_2} \times$ ♂ $\frac{yp_2}{yp_2}$	92	16	33	90	49	21.2
♀ $\frac{yp_2}{yp_2} \times$ ♂ $\frac{YP_2}{yp_2}$	96	25	19	84	44	19.6
Total	188	41	52	174	93	20.4

studies with the mouse, showing crossing over to be a little less frequent in the male than it is in the female.

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OFFSPRING OBTAINED FROM MALES REARED AT DIFFERENT TEMPERATURES IN HABROBRACON

THE parasitic wasp *Habrobracon juglandis* (Ashmead) normally produces females (biparental) from fertilized eggs and males (impaternate) from unfertilized eggs. If parents are closely related, however, many of the fertilized eggs fail to develop, others produce females and a minority give rise to biparental males.

Females which had been mated to males reared at a constant temperature were kept at different temperatures during development and fertilization of the eggs. The offspring from the cool temperature group showed a higher percentage of biparentals, but fewer of these were males (P. W. Whiting and Russell L. Anderson, 1932). The experiments reported herewitb show

what happens when the males (stock 11) are reared at different temperatures, while females of a closely related stock (11–o) are kept at a constant temperature (30° C.). Eye color served as the criterion of biparentalism. The male parents had the dominant black eye color, the female parents the recessive orange. Biparental sons and daughters were therefore black, while the impaternate sons (from unfertilized eggs) were orange.

The males were reared at three different temperatures. Some were kept at 20° C. throughout life, others at 30° C. The 36° C. group were started at 30° C. in order to avoid sterilizing effect of the higher temperature on the mothers. After developing for four days or less, the males were transferred so that sperm were produced at 36° C.

The pairs were set and left together for eighteen hours at room temperature. Matings were not observed. Biparental offspring appeared in 97 (61.4– per cent.) of the 158 pairings of 36° C. males: in 128 (78.5+ per cent.) of the 163 pairings of 30° C. males: and in 82 (86.3+ per cent.) of the 95 pairings of 20° C. males. These figures indicate effect of temperature on mating activity of males, change to room temperature reducing activity of the high temperature group but increasing activity of the low temperature group.

Of the 307 pairings in which matings had occurred, as shown by production of biparental offspring, only eleven failed to produce biparental males. Ten of these, scattered through the three temperature groups, included less than four females each, so that they are of no significance. The eleventh, however, belonging to the group in which male parents were reared at cool temperature, included, besides 21 impaternate males, 83 biparental offspring, all of which were females. All other large fraternities contained several biparental males so that this case is definitely exceptional.

Table I summarizes data according to vials (A, B, C, D, E) through which mothers were successively transferred. Later vials therefore indicate increasing age of mothers and increasing time since mating, with consequent increase in age and reduction in quantity of sperm. As soon as a female ceased giving biparental offspring, her impaternate sons were no longer included in the summaries. Nevertheless, for each experiment it may be seen that there is in general a decrease in percentage of biparentals (biparentals $\times$ 100/total), as sperm become depleted.

TABLE I

FRATERNITIES SUMMARIZED ACCORDING TO TEMPERATURES AT WHICH FATHERS WERE REARED AND ACCORDING TO VIALS THROUGH WHICH MOTHERS WERE SUCCESSIVELY TRANSFERRED

Temperatures	Vials	Biparental ♀♀	Biparental ♂♂	Impaternate ♂♂	Biparental ♂♂ × 100 / Biparentals	Biparentals × 100 / Total
36° C.	A	707	207	450	22.65	67.01
	B	568	155	599	21.44	54.69
	C	629	214	600	25.39	58.42
	D	323	124	464	27.74	49.07
	E	392	124	348	24.03	59.72
	Total	2,619	824	2,461	23.93 ± 0.49	58.32 ± 0.43
30° C.	A	579	159	376	21.54	66.25
	B	1,282	443	802	25.68	68.26
	C	929	337	775	26.62	62.03
	D	514	239	489	31.74	60.63
	E	487	158	385	24.50	62.62
	Total	3,791	1,336	2,827	26.06 ± 0.41	64.46 ± 0.36
20° C.	A	712	203	581	22.19	61.16
	B	682	242	590	26.19	61.03
	C	544	172	482	24.02	59.77
	D	365	135	359	27.00	58.21
	E	189	55	201	22.54	54.83
	Total	2,492	807	2,213	24.46 ± 0.50	59.85 ± 0.45

There is a significant deficiency in percentage of biparental offspring associated with rearing of males either at the higher (difference = 6.14 ± 0.56 per cent.) or at the lower (difference = 5.61 ± 0.58 per cent.) temperature. Of the three temperatures, 30° C. appears to be the best for production of sperm.

Among the offspring from fertilized eggs, there are more males developed (biparental ♂♂ × 100/biparentals) in vials D (29.30 ± 0.84 per cent.) than in vials A (22.16 ± 0.55 per cent.) (difference = 7.14 ± 1.00 per cent.) or in vials E (23.63 ± 0.76 per cent.) (difference = 5.67 ± 1.13 per cent.). For each experiment the difference in this respect between vials A and D is significant, but between vials D and E totals only are significant, although the reduction appears for each group. The percentage of males among biparental offspring therefore at first increases but later decreases with aging of the sperm and with advancing age of the mothers.

Among the offspring developing from fertilized eggs there are more males when sperm are produced at 30° C. than at 36° C. (difference = 2.13 ± 0.64 per cent.) or at 20° C. (difference = 1.60 ± 0.65 per cent.).

The percentage of males among the biparentals was calculated for each of the larger fraternities (those containing not less than eighteen biparentals) and frequency distributions were made of these percentages for each of the temperature groups. It may be seen (Table II) that in each case the array of fraternities is

TABLE II

FREQUENCY DISTRIBUTIONS OF PERCENTAGES OF MALES AMONG BIPARENTALS FOR FRATERNITIES SIRED BY MALES REARED AT DIFFERENT TEMPERATURES

Temperatures		Percentages of males among biparentals									
		1	10	15	20	25	30	35	40	45	Total
36° C.	Fraternities number	1	7	4	16	25	15	7	4		79
	per cent.	1.3	8.9	5.1	20.0	31.7	19.0	8.9	5.1		100
30° C.	Fraternities number		5	11	24	30	27	13	4	1	115
	per cent.		4.4	9.6	20.8	26.1	23.4	11.3	3.5	0.9	100
20° C.	Fraternities number		5	10	14	17	13	10	4	1	74
	per cent.		6.8	13.5	18.9	23.0	17.6	13.5	5.4	1.3	100
Total	Fraternities number	1	17	25	54	72	55	30	12	2	268
	per cent.	0.4	6.3	9.3	20.1	26.9	20.5	11.2	4.5	0.8	100

unimodal with approximately the same mode. However, the fraternities sired by males reared at the highest temperature showed the closest grouping about the mode, while the fraternities from the males reared at cooler temperatures showed increasing deviation from the mode.

According to the theory of sex-determination recently advanced for Habrobracon (P. W. Whiting, 1933), females are digametic, X/Y, while impaternate males are either X or Y. Females are formed by heterosyngamy (fertilization of an X egg nucleus with a Y sperm or the reverse): biparental males, XX or YY, are formed by homeosyngamy (fertilization of an X egg nucleus with an X sperm or of a Y with a Y). Since all sperm from any one male are similar, all fertilized eggs from any one

pairing are fertilized by one type of sperm, but differential maturation occurs in the egg so that heterosyngamic, XY or YX, combinations take place more frequently than homeosyngamic, XX or YY, the latter failing altogether when parents are unrelated.

The differences in percentages of biparental offspring and of males among biparentals herewith reported are probably not caused by conditions affecting relative viability of heterosyngamic, homeosyngamic and unfertilized eggs, but rather by such conditions as affect percentage of eggs fertilized or amount of differential maturation. It is idle at this time to speculate as to what these conditions may be since several alternatives may be suggested.

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A FURTHER STUDY OF THE EFFECT OF TEMPERATURE ON CROSSING OVER

INTRODUCTORY

THAT different parts of a given chromosome vary considerably in their susceptibility to environmental effects upon crossing over has been known for some time. Muller (1925), using a 3rd chromosome stock, *ru h st p ss e*, showed that the effect of light dosages of x-rays (26.8 Holzknecht units) was apparent only in the scarlet-pink region; that double this dosage extended the effect to the regions adjacent to scarlet-pink. Bridges (1929), using a similar stock, $\frac{\text{ru}\quad \text{D}\quad \text{p}^{\text{p}}}{\text{h}\quad \text{st}}\ \frac{\text{e}^{\text{s}}}{\text{ss}}$, showed that the effect of age was similarly concentrated in this scarlet-peach region. Plough (1917), using the stock, *se ss e*^{s} *ro*, was able to demonstrate that the right-hand third of the chromosome was unaffected by an increase in temperature, whereas the middle third showed significant increase. Inasmuch as the experiments with x-rays and age indicate susceptibility to be concentrated in an

extremely small area adjacent to the point of spindle fiber attachment, it was felt worthwhile to extend the work of Plough (1921) so as to test out the left-hand area of the chromosome from 0.0 to 26.0, and further to subdivide the middle third of the chromosome so as to delimit the scarlet-pink (peach) region. A stock was used containing the characters roughoid, hairy, scarlet, pink, spineless, sooty: *ru h st p ss* e^s.

EXPERIMENTAL

Individuals of the mutant stock were bred in the conventional manner and mated with pure wild-type individuals; the heterozygote F_1 was backcrossed to the mutant stock and the resultant offspring examined and tabulated. In each experiment (*i.e.*, at 25 C. and 31.5 C.) all stocks and generations were kept constantly within 0.5 C. of the indicated temperature, except during the necessary minutes required for transfer of flies from one bottle to another. A summary of the data is given in Table I.

TABLE I

Region	Recombination at 25 C.	Recombination at 31.5 C.	Amount increase	Per cent. increase
Roughoid-Hairy	24.36	21.35	.....	.....
Hairy-Scarlet	18.80	22.25	3.45	17
Scarlet-Pink	3.68	14.43	10.75	292
Pink-Spineless	7.42	12.28	4.86	65
Spineless-Sooty	10.30[1]	10.17	.....	.....
Total number flies	3,691	2,557		

[1] Classification of sooty was unsatisfactory in the original experiment. Repetition with 736 flies classified for spineless and sooty only gave a value of 13.3, including 157 flies classified as doubtful. Omitting these, the value is as recorded. Classification in the high temperature experiment is believed correct.

These data bring the effects of temperature thoroughly in line with those reported for age and x-rays: *i.e.*, no effect on the terminal thirds of the chromosome; the effect on the middle third being concentrated in the central region scarlet-pink and extending to the adjacent regions. This concentration in the central region was foreshadowed by a preliminary experiment of Plough's, in which, by inserting Dichaete between sepia and

spineless, he found the increase between sepia and Dichaete to be approximately 50 per cent. in contrast to over 100 per cent. increase between Dichaete and spineless.

Even in the light of Plough's preliminary experiment, the magnitude of the increase in the central region demanded further check. In the first place, there was the possibility of modifying factors in one or the other of the stocks. This possibility was checked mathematically by calculating from our data the recombination value between hairy and spineless *as if the intervening genes were not present.* This figure is practically comparable to Plough's recombination value for the area sepia-spineless, inasmuch as one terminal (spineless) is common to both and the other (hairy in the one, sepia in the other) deviates by only 0.5 unit on the Morgan, Bridges, Sturtevant map. The comparison is given in Table II.

TABLE II

	Recombination at low temperature	Recombination at high temperature	Difference	Per cent. difference
Plough	27.4[2]	36.6	9.2	33
Schwab	25.7	36.5	10.8	42

[2] Calculated from Plough's data on the first seven days of his count.

Because the differences shown in Table II are not statistically significant, the phenomena studied in the two stocks are probably the same.

As a further experimental check, a variation of the original experiment was made as follows: Pairs of mutant and wild-type flies were taken from the mass-mated laboratory stock and used in the same way as the flies derived by intensive brother-sister matings were used in the first experiment. The resultant offspring were tabulated only for scarlet and pink, thus allowing extremely careful identification. The results are compared with parallel data from the first experiment in Table III.

Subsequent to the completion of this work, our attention was called to a paper by Kirssanow (1931) reporting his results with temperature and x-rays on a similar multiple stock, *ru h st* p^p *ss* e^s. Although his results are qualitatively in line

TABLE III

	Recombination at 25 C.	Recombination at 31.5 C.	Difference	Per cent. difference
1st Experiment	3.68	14.43	10.75	292
2nd Experiment	3.12	12.96	9.84	315
S. Deviation	0.37	1.00		
Total no. flies in 2nd Experiment	2,209	1,126		

with those reported here (*i.e.*, significant change concentrated in the scarlet-peach region) there is a strong quantitative contrast which is summarized in Table IV.

In short, Kirssanow tested the effect of each factor alone; of the two factors together in the order x-ray and then temperature; and together in the order temperature and then x-ray. Compared to our data for the third chromosome, to Plough's data for temperature on the second chromosome, to Bridges' data for age on the third chromosome, and to the data of Mavor and Svenson (1924) for x-rays, these values of Kirssanow's are extraordinarily low, for in each of these other papers the recombination value under the experimental conditions is reported as approximately triple that under control conditions.

Kirssanow states that in each case the data are based on counts from the seventh to the twelfth day. This fact assumes significance with reference to the contradiction between his data and the other data cited, for Plough has definitely shown that the effect of temperature upon recombination is extremely critical, not only in time of origin but in duration, and Mavor and Svenson have demonstrated a definite time curve for x-rays. Specifically, Plough has shown that the effect of temperature

TABLE IV

Kirssanow "Control"	3.0
Schwab, 25 C. (Mean)	3.4
Kirssanow, 31 C.	4.9
Schwab, 31.5 C. (Mean)	13.7
Kirssanow, x-ray	5.6
Kirssanow, ray, then 31 C.	12.6
Kirssanow, 31 C., then ray	9.1

upon crossing over is not apparent until seven days after treatment, and that, moreover, the effect lasts approximately only as long as did the treatment. Mavor and Svenson have shown that the effect of x-rays on crossing over does not appear until the sixth day, has its peak from the seventh to the ninth days inclusive and thereafter subsides. If, then, one submitted Drosophila to high temperature, then ceased this treatment in order to subject them to x-rays, a count of the resultant offspring from the seventh to the twelfth days (presumably with reference to first emergence) could not possibly represent the peak effect of both, or perhaps of either, since both appear at approximately the same interval after initiation of treatment and the time of treatment with each was necessarily different. Indeed, it is possible that only a small fraction of the five days' count of flies was really heat-affected, depending, of course, entirely on the duration of the heat treatment; and certainly a five-day count of the result of a treatment with a three-day peak (x-rays) must necessarily be lower than the value arrived at by a count corresponding with the peak. The same criticism holds if one reverses the order; submitting the flies first to x-rays and then to temperature; for again the curves of the two, plotted on a time line, can not be congruent with reference either to origin or shape. Finally, in an experiment using temperature alone, a five-day count from the seventh to the twelfth day after emergence would not be a test of the maximal effect of temperature unless care were taken to see that the duration of treatment was as long or longer than the duration of the count, and, moreover, that the treatment was made at the appropriate time with reference to the time of count.

Unfortunately, the criticisms implied above must remain in the sphere of the "possible" or "probable," rather than be definitely applied to Kirssanow's report, for he may have taken into account all or some of the facts cited. Whether he did or not, however, must remain an open question, inasmuch as nowhere in his paper could be found any statement of the exact time of treatment with x-rays or temperature in his various experiments, nor of the duration of his temperature treatments.

Summary

(1) The effect of an increase of temperature upon recombination in the third chromosome is found to be concentrated between

scarlet and pink (the area of spindle fiber attachment) and spreading with high decrement to the adjacent regions. These results are in agreement with those reported by Muller for x-rays and by Bridges for age.

(2) Quantitatively contradictory data reported by Kirssanow may possibly be due to insufficient attention to the time element involved in x-ray and temperature effects.

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